HENRY FORD HOSPITAL

International Symposium

Hepatitis Frontiers

The symposium was sponsored by the Henry Ford Hospital
Detroit Michigan and held at the hospital October 25 26 27 1956

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HENRY FORD HOSPITAL INTERNATIONAL SYMPOSIUM

Hepatitis Frontiers

Editors

FRANK W HARTMAN MD

Medical Research Adviser Director of Professional Services
Office of the Surgeon General USAF Washington DC
formerly Director of Laboratories Henry Ford Hospital

GERALD A LoGRIPPO MD

Associate in Charge Division of Microbiology
Department of Laboratories Henry Ford Hospital

JOHN G MATTER MD

Physician in Chief Department of Medicine Henry Ford Hospital

JAMES BARRON MD

Associate Surgeon Division of General Surgery Henry Ford Hospital



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Participants in the Formal Program

J GARROTT ALLEN MD
Professor of Surgery William H Danforth Laboratory for Research in Surgery University of Chicago Chicago Illinois

HARLAN DWIGHT ANDERSON PH D
Associate Director Division of Laboratories Michigan Department of Health Lansing Michigan

ARCHIE HERBERT BAGGENSTOSS MD
Section of Pathologic Anatomy Mayo Clinic and Mayo Foundation Rochester Minnesota

FREDERICK B BANG MD
Professor of Pathobiology School of Public Health Associate Professor of Medicine School of Medicine Johns Hopkins University Baltimore Maryland

JAMES BARRON MD
Associate Surgeon Division of General Surgery Henry Ford Hospital Detroit Michigan

CHARLES HERBERT BEST MD
Professor of Physiology University of Toronto Toronto Canada

MOGENS BJØRNEBOE MD
Blegdamshospital Copenhagen Denmark

HENRY L BOCKUS MD
Professor of Medicine Graduate School of Medicine University of Pennsylvania Philadelphia Pennsylvania

JESSE L BOLLMAN MD
Section of Biochemistry Mayo Clinic and Mayo Foundation Rochester Minnesota

GERRIT BRAS MD
Department of Pathology University of British West Indies Jamaica British West Indies

RALPH W BRAUER PH D
Head Pharmacology Branch U.S Naval Radiological Defense Laboratory San Francisco California

THOMAS C CHALMERS MD
Chief of Medicine Lemuel Shattuck Hospital Clinical Associate in Medicine Harvard Medical School Boston Massachusetts

GILBERT DALLDORF MD
Director of Laboratories and Research State of New York Department of Health Albany New York

CHARLES S DAVIDSON MD
Thorndike Memorial Laboratory and Second and Fourth (Harvard) Medical Services Boston City Hospital Associate Professor Department of Medicine Harvard Medical School Boston Massachusetts

HECTOR DUCCI M D
Section A of Medicine University of Chile Medical School Hospital del Salvador Santiago Chile

EDWARD A GALL M D
Professor of Pathology College of Medicine University of Cincinnati and the Cincinnati General Hospital Cincinnati Ohio

SVEN GARD M D
Department of Virus Research Karolinska Institute Medical School State Bacteriological Laboratory Stockholm Sweden

ROSS L GAULD M D
Chief Department of Epidemiology Walter Reed Army Institute of Research Walter Reed Army Medical Center Division of Preventive Medicine Washington D C

SAM T GIBSON M D
Medical Director Blood Program American National Red Cross Washington D C

PAUL GYORGY M D
Professor of Pediatrics School of Medicine University of Pennsylvania Philadelphia Pennsylvania

FRANK W HARTMAN M D
Medical Research Adviser Director of Professional Services Office of the Surgeon General U.S.A.F., Washington D C

W PAUL HAVENS Jr M D
The Jefferson Medical College Philadelphia Pennsylvania

KENNETH R HILL M D
Professor of Pathology University of British West Indies Jamaica British West Indies

CHARLES JOHNSTON M D
Professor of Surgery Wayne University Medical School Detroit Michigan

RUSSELL T JORDAN M D
Chairman Department of Microbiology Division of Research City of Hope Medical Research Institute Duarte California

ANNETTA R KELLY Ph D
Associate in Pharmacology Henry Ford Hospital Detroit Michigan

LLOYD L KEMPE, Ph D
Associate Professor Department of Bacteriology, University of Michigan Medical School Ann Arbor Michigan

SAUL KRUGMAN M D
Department of Pediatrics New York University College of Medicine New York New York Willowbrook State School Staten Island New York

HENRY G KUNKEL M D
The Rockefeller Institute for Medical Research New York New York

GERALD A LoGRIPPO M D
Associate in Charge Division of Microbiology Henry Ford Hospital Detroit Michigan

FREDERIC OGDEN MacCALLUM, M.D.
Virus Reference Laboratory, Central Public Health Laboratory, London, England

NOEL FRANCIS MACLAGAN, M.D.
Professor, Department of Chemical Pathology, University of London, London, England

G. ADOLPH MARTINI, M.D.
I. Medical Klinik, Universitat Krankenhaus, Hamburg-Eppendorf, Germany

JOHN G. MATEER, M.D.
Physician in Chief, Department of Medicine, Henry Ford Hospital, Detroit, Michigan

J. WILLIAM McLEAN, Jr., M.D.
Research Division, Parke, Davis and Company, Detroit, Michigan

JOSEPH L. MELNICK, Ph.D.
Yale University School of Medicine, Section of Preventive Medicine, New Haven, Connecticut

DWIGHT J. MULFORD, Ph.D.
Professor, Department of Biochemistry, University of Kansas, Lawrence, Kansas

RODERICK MURRAY, M.D.
Director, Division of Biologics Standards, National Institutes of Health, Washington, D. C.

JOHN R. NEEFE, M.D.
6 7 Eleventh Street North, St. Petersburg, Florida

ROBERT F. NORRIS, M.D.
Director, William Pepper Laboratory of Clinical Medicine, University of Pennsylvania, Philadelphia, Pennsylvania

JOHN R. PAUL, M.D.
Professor of Preventive Medicine, Yale University School of Medicine, New Haven, Connecticut

ROBERT B. PENNELL, Ph.D.
Director, Protein Foundation Laboratories, Jamaica Plain, Massachusetts

ERNEST C. POLLARD, Ph.D.
The Josiah Willard Gibbs Research Laboratories, Yale University, New Haven, Connecticut

HANS POPPER, M.D.
Cook County Hospital, Chicago, Illinois

ARON M. RAPPAPORT, M.D., Ph.D.
Associate Professor of Physiology, University of Toronto, Toronto, Canada

JOHN G. REINHOLD, Ph.D.
William Pepper Laboratory of Clinical Medicine, University of Pennsylvania, Philadelphia, Pennsylvania

CLARENCE E. RUPE, M.D.
Physician in Charge, Medical Clinic No. 4, Henry Ford Hospital, Detroit, Michigan

VICTOR M. SBOROV, M.D.
945 Middlefield Road, Redwood City, California

LEON SCHIFF M D PhD
Professor of Clinical Medicine University of Cincinnati College of Medicine Cincinnati Ohio

SHEILA SHERLOCK M D
Department of Medicine University of London London England

HANS F SMETANA M D
Armed Forces Institute of Pathology Washington D C Patel Chest Institute Delhi University Delhi India

JOSEPH STOKES Jr M D
Professor of Pediatrics University of Pennsylvania Philadelphia Pennsylvania

WILLIAM S TILLETT M D
Professor of Medicine New York University College of Medicine New York New York

JOHN G TRUMP D Sc
Professor of Electrical Engineering Massachusetts Institute of Technology Cambridge Massachusetts

G VIOLLIER M D
Medizinische Universitats klinik Basle Switzerland

R VISWANATHAN M D
Deputy Director General of Health Services Government of India New Delhi India

ROBERT WARD M D
Department of Pediatrics New York University College of Medicine New York New York

CECIL J WATSON M D
Professor of Medicine University of Minnesota School of Medicine Minneapolis Minnesota

THOMAS H WELLER M D
Professor of Tropical Public Health Harvard University School of Public Health Boston Massachusetts

FELIX WROBLEWSKI M D
Medical Service and Sloan Kettering Institute Memorial Center for Cancer and Allied Diseases Cornell University Medical College New York New York

Discussants

A H Baggenstoss
Rochester Minnesota

M Bjørneboe
Copenhagen Denmark

Jesse L Bollman
Rochester Minnesota

Ralph W Brauer
San Francisco California

T C Chalmers
Boston Massachusetts

Gilbert Dalldorf
New York New York

Charles S Davidson
Boston Massachusetts

Hector Ducci
Santiago Chile

Alfred S Evans
Madison Wisconsin

William W Faloon
Syracuse New York

Thomas Francis
Ann Arbor Michigan

Edward A Gall
Cincinnati Ohio

Sven Gard
Stockholm Sweden

Kenneth R Hill
Jamaica British West Indies

Karol A Hok
Berkeley California

Charles Johnston
Detroit Michigan

Russell T Jordan
Duarte, California

Leo Krainer
Washington D C

Henry G Kunkel
New York New York

Gerald A LoGrippo
Detroit Michigan

Walter N Mack
East Lansing Michigan

Noel F Maclagan
London England

G A Martini
Hamburg Germany

James W Mosley
Atlanta Georgia

John R Neefe
St Petersburg Florida

Robert F Norris
Philadelphia Pennsylvania

John R Paul
New Haven Connecticut

Ernest C Pollard
New Haven Connecticut

Hans Popper
Chicago Illinois

A M Rappaport
Toronto Canada

John G Reinhold
Philadelphia Pennsylvania

Andrew Sass Kortsak
Toronto Canada

Victor M Sborov
Redwood City, California

Leon Schiff
Cincinnati Ohio

Sheila Sherlock
London England

Hans F Smetana
Delhi India

Joseph Stokes Jr
Philadelphia Pennsylvania

Cyril Stulberg
Detroit, Michigan

Allen Taylor
Detroit, Michigan

John G Trump
Boston, Massachusetts

Robert Ward
New York New York

Cecil Watson
Minneapolis Minnesota

Thomas H Weller
Boston, Massachusetts

Felix Wroblewski
New York, New York

Foreword

In the past few years a number of timely and interesting symposia have been sponsored by the Henry Ford Hospital These have been on an international level bringing together from many parts of the world students of the subject under discussion The symposia have made an unusually significant contribution to progress in various areas of medical research This has been due in part to a fortunate choice of subject matter and to the participation of many well qualified scholars but the character of the symposia their freedom of discussion and interchange of ideas and new information has been the primary factor in their success

It would indeed be difficult to conceive of any topic more appropriate than viral hepatitis for such a symposium the transactions of which are recorded in the present volume This subject touches intimately on a great many disciplines in the medical sciences including among others the anatomy and physiology of the liver its more tangible functions and methods of measuring their aberration in disease especially viral hepatitis the pathology of hepatitis and the fascinating and controversial question of its importance in relation to chronic liver disease especially cirrhosis clinical diagnosis and treatment and of paramount importance virology and epidemiology In World War II as in previous wars there was a striking increase in morbidity incidence of the disease but its viral etiology was now recognized for the first time and genuine forward progress was begun Nevertheless infectious hepatitis is probably the most important of the viral diseases yet unconquered at least insofar as any useful concentration of the virus is concerned Thus the present symposium was especially timely in that some current important advances in this area were described

The wide range of subtopics undoubtedly contributed greatly to the general interest and the feeling that the symposium was soundly conceived and its program well planned All who attended will be grateful to the planning committee and will appreciate the generous sponsorship of the Henry Ford Hospital and its staff and all who have an opportunity to read these transactions will be grateful to the publisher Little Brown and Company for assuming the financial responsibility for publication

Cecil J Watson M D

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PART I

Anatomy, Physiology and Pathology of the Liver

Moderator CHARLES H BEST M D (*Toronto Canada*)

I

*The Structural and Functional Acinar Unit of the Liver — Some Histopathological Considerations**

A. M RAPPAPORT MD PH D

(Toronto Canada)

The liver is perhaps the organ most hailed for its various functions but amazingly the least understood as to the microscopic structure of its units that carry out these multiple functions In recent times there even has been a tendency to present this organ as an indivisible mass This can only mean failing to see the trees for the woods On the other hand one would be mistaken in assuming that the delimitation of structural and functional units in the liver results in substitution of a mosaic for organic unity May I remind you that in other organs the understanding of their function has been advanced by the description of their structural and functional units Renal physiology for example is based on the structural concept of the nephron which is also a functional unit

I will attempt to present the structural units of the liver with respect to their circulatory secretory and metabolic function in the hope that future research may succeed in measuring their circulatory and metabolic activity Also the pathology of such units will be sketched

The structural and functional unit of the liver has been called by us liver acinus a name given already by Malpighi (1666) [1] and later by Mascagni [2] to the smallest amount of hepatic parenchyma attached to the smallest branches of afferent vessels and bile ducts isolated by teasing the tissue In our microscopic study we too have adopted the terminal branches of portal vein hepatic artery and bile duct branching out from the smallest triangular portal field as the axis around which are organized small microscopic clumps of hepatic tissue *irregular* in size and shape This trio of associated channels presents the dynamic line along which nutrients and oxygen are moved into and the secretory product — the bile — is moved out from the parenchymal clump The existence of such units has been proved [3] by simultaneous injection of two differently

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FIGURE 1 Subdivision of the hexagonal lobule into hepatic structural units The structural units are small berry like parenchymal masses situated around the trio of terminal branches of portal vein hepati artery and bile duct bran hing out from a small portal space The structural unit is the horizontal section thr ugh a red colored hepatic unit extending from one central vein t another (From Rappaport Borowy Lougheed and Lotto *Anat Rec* 1954)

colored gelatin masses through the two main branches of portal vein hepatic artery or bile duct Differently colored microscopic areas are seen centered around their axial trio of channels and extending towards at least two neighboring terminal hepatic (central) veins they occupy sectors only of the adjacent hexagonal fields (Figure 1)

In this slide of a rabbit s liver injected by our technique (Figure .) you can see the section of an acinus with the axial vessels in its center and the terminal hepatic (central) veins at the periphery In the human liver the injection of India ink under 10 mm Hg pressure permits the



FIGURE 2 Acinar unit in the liver of a rabbit The area clear of India ink is centered around the axial channels that grow out from a small portal space It extends into two adjacent hexagonal fields the central veins of which are seen in the lower left and upper right corners (Thick cleared section × 65 approx.) (From Rappaport Borowy Lougheed and Lotto *Anat Rec* 1954)

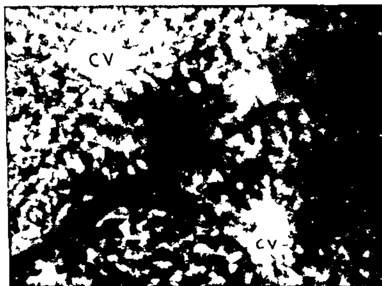


FIGURE 3 Liver acinus in human liver The acinus occupies sectors only of two adjacent hexagonal fields and reaches their central veins The axial terminal portal branch of the structural unit is injected with India ink and runs perpendicularly to the two hepatic (central) veins with which it interdigitates It is visualized by a fortunate cut parallel with almost its entire length The central veins lie close to each other in this section (Thick cleared section × 200) (From Rappaport Anatomic Considerations In *Schiff L (ed.) Diseases of the Liver* p 9)

visualization of the acini (Figure 3) They stand out because of the variation in filling with dye The coloring is heavier when the plane of section is closer to the axial channel bringing in the dye As the acini vary in shape they are cut at various levels by the same plane of section Each acinus is of course part of a larger clump of tissue — a complex acinus arranged around a trio of preterminal channels which divide into the tridimensionally spread out terminal branches supplying the simple acinus (Figures 4 and 5)[4]

The complex acini are parts of larger clumps acinar agglomerates The latter are arranged around larger branches of portal vein hepatic artery and bile duct (Figure 6) Acinar agglomerates are parts of a group of agglomerates supplied and drained by still larger vascular and biliary branches of higher anatomical order A number of such groups of acinar agglomerates will form a lobe in the multilobulated liver of an animal The transition from the microscopic to the macroscopic level occurs in the grouped acinar agglomerates Thus the microscopic units do not stay isolated but are naturally integrated into larger units of which they are part and which they help to form Following the path of the divisions of afferent vessels and bile duct along which the liver has developed from an outgrowth of the foregut one can give a correct description of hepatic structure The genesis of the composite hexagonal pattern is fully explained by the tridimensional branching out of bile ducts and afferent vessels in a limited space under the cupola of the diaphragm and their interdigitation with the hepatic venous branches[3]

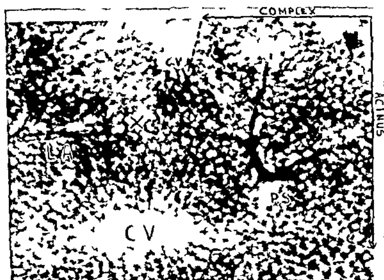
A closer look at the simple acinus reveals a zonal relationship between its cells and their blood supply The liver cells situated close to the axial vascular twigs (Figure 7 Zone 1) are the first to be supplied with blood hence their blood supply is rich in oxygen and nutrients The more distant the cells are from the site where the terminal portal and arterial twigs empty into the sinusoids the poorer the quality of blood that bathes them and the less their resistance to damage (Zones and 3) It is evident that not all cells lying in the area concentric about the portal space share equally the possibility of being supplied with fresh blood although this is generally assumed Some cells in Zone B or C further from the portal field will have an excellent blood supply from the terminal vascular twigs branching out from the same portal space Some cells in Zone A although *close to the portal triad are remote from the arborization of the terminal* afferent vessels and therefore at a disadvantage with regard to oxygen and nutrients This zonal dependence becomes evident in certain abnormal metabolic nutritional and circulatory states which might leave permanent pathologic imprints on the structure of the acinar clump

Starvation of a rat or mouse for 4 hours causes transient fat accumulation in Zone 1 (Figure 8) The fat mobilized from the depots is brought



C. A ompl us P pr t rmn l port l b a ch

FIGURE 4 Human liver injected with India ink through portal vein (Thick cleared section × 15)



P S p rt l p c C. V e t l

FIGURE 5 Human liver injected with India ink The complex ac nus is form d by tl ree mple ac ni branch ng out trid mensionally from the portal space Of these the left one meets with its cap llarized tip the capillaries of a neigh boring simple ac nus (L¹A¹) taking part in the formation of a hexagonal field X is the weak circulatory point where lesions break through from one he g nal field to the neigl boring one l nking their central ve ns (Th ck cleared section × 65)



FIGURE 6 Human acinar agglomerate Human liver injected with India ink through the portal vein (Thick cleared section × 15)

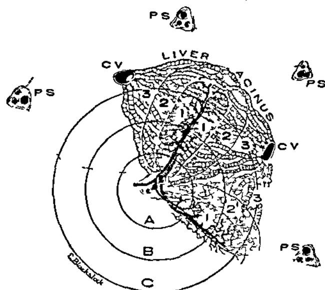


FIGURE 7 The blood supply of the hepatic acinar unit The liver acinus occupies adjacent sectors of neighboring hexagonal field Zones 1 2 and 3 respectively represent areas supplied with blood of first second and third quality with regard to oxygen and nutrient These zones center about the terminal afferent vascular branches and extend into the portal field from which these branches originate Zones 1' 2' and 3' designate corresponding areas in a portion of an adjacent acinar unit In Zone 1 and 1' the afferent vascular twigs empty into the sinusoids. The circles A B and C delimit concentric bands of hepatic parenchyma around a small portal field They mark the circulatory zones in decreasing order of nutrients and oxygen content as assumed in the classical concepts of hexagonal units (From Rappaport Borowy Lougheed and Lotto *Anat Rec* 1954)



C V entr l e P S port l sp c

FIGURE 8 Liver of a normal mouse starved for 24 hours The fat (black) is accumulated in Zones 1 and 2 close to the afferent vessels branching out from the portal space (Frozen section Oil Red O (ORO) stain × 130 approx)



C V t l v P S port l p c

FIGURE 9 Liver of rat in chol ne deficiency for 32 days Note the fat (black) accumul ted in Zone 3 It utl nes a l er ac nus the axial structures (portal vein b le ductule) of wh ch branch out from a small portal space (Frozen section ORO sta n × 40)



Figure 10 Liver of a rat in choline deficiency for 3 days The fat (black and vacuoles) occupies Zones 3 and outlines the various acinar clumps that differ in size and shape Note the bands of fatty tissue linking neighboring central veins They pass through the weak circulatory points (V) where the tips of Zones 3 of adjacent acini meet (Frozen section ORO stain × 15)

into the liver and is found so to speak in the freight yard It has been unloaded in Zone 1 where it is offered to the liver cells for metabolism

Choline deficiency for 3 days a nutritional anomaly produces fat accumulation in Zone 3 while Zones 2 and 1 show no pathologic change (Figure 9) Note please that Zone 3 extends down to the small portal space and that the same occurrence in several acini adjacent to this portal field may produce the image of periportal fat, although the fat is accumulated in a peripheral circulatory zone A lower magnification of the same field (Figure 10) illustrates that the path along which the fat spreads crosses another weak and peripheral point of circulation It is the site where the capillarized tips of the acinar axial vessels meet Here occurs the breakthrough of the pathologic process from one hexagonal field into the other The linking of one central vein to the other by fatty and later by fibrous bands is thus explained without difficulty

When because of choline deficiency the fat has permanently damaged the parenchymal cells it is replaced by fibrous membranes of the same zonal arrangement The fibrous bands situated at the shriveled periphery of the liver acini clearly delineate them from each other (Figure 11) The so-called pseudolobulation thus produced reveals the true acinar lobules each of which, however bears the marks of fibrosis (Figure 12)

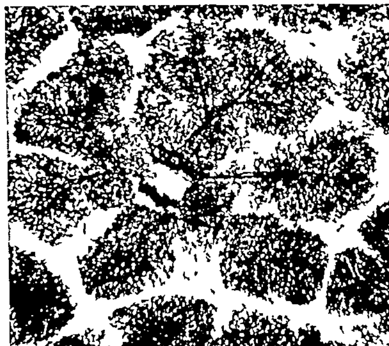


FIGURE 11 Liver The structural units have been dissected by the formation of fibrous trabeculae in this preparation from a rat in the early stages of dietary cirrhosis due to choline deficiency A large conducting branch of the portal vein occupies the center of the field From it radiate a number of terminal portal venules each surrounded by a pyriform mass of sinusoids supplying parenchyma which is intact except at the periphery of each acinus Branches of the central vein are situated at the periphery of the field and are linked by an annular ring of fibrous tissue from which other trabeculae extend along paths midway between the terminal venules toward the conducting branch of the portal vein in the center of the field In two places the fibrous tissue has extended to the adventitial sheath of the conducting vessel Fibrous trabeculae appear relatively free of injected ink (100μ thick cleared section $\times$ 80)

Severe anoxia induced experimentally in the dog by ligation of the common hepatic artery 30 hours after the formation of an Eck fistula regularly causes necrosis in Zones 3 and 2 in a pattern similar to that of fatty change and fibrosis (Figure 13)

Note again that the strands of necrosis in the hexagonal fields run along Zone 3 and break through the peripheral capillarized tips of adjacent acini linking central veins to central veins (Figure 14) Another example of damage to the acini is that done by chronic hepatitis Although it results from an acute irregular or as Smetana[5] calls it kaleidoscopic transformation of the normally quiescent liver parenchyma still some lesions

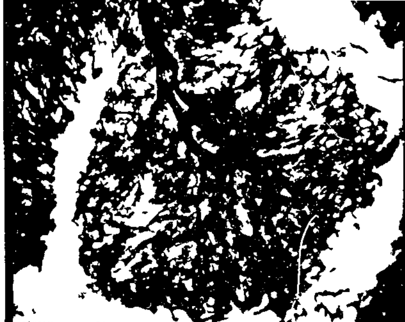


FIGURE 12 Higher magnification of a shriveled structural acinar unit seen in Figure 11 Note how the fibrous bands block the passage of ink through the few remaining narrowed outlet venules The increased resistance in the small vessels of the peripheral vascular bed is a cause of portal hypertension (100μ thick cleared section $\times$ 240)



C V t l P S pet l pc

FIGURE 13 Liver of a dog Severe ischemia has caused necrosis in Zone 3 and in part of Zone 2 The band of necrosis has disconnected two adjacent acini (upper left corner) by breaking through the tips of their Zones 3 it links the central veins to each other (Frozen section ORO stain $\times$ 65 approx)



FIGURE 14 The breakdown of the uniform parenchymal mass into its acinar constituents Ischemic necrosis developing and progressing along the periphery of the acinar units delineates their boundaries Note portal spaces (1 2 3) situated in the 3 corners of the field and arranged almost equidistantly on a circle surrounding the central vein Note also the irregular acini (injected with India ink) that grow out from the portal spaces and a small ink filled venule leaving space 3 The central necrosis has stellate shape and its projections extend toward and beyond the weakest points of circulation the site where the capillarized tips of the acini meet each other A similar distribution of lesions is observed in fatty change and in dietary cirrhosis (From Rappaport Anatomic Considerations In Schiff L (ed) *Diseases of the Liver* p 15)

may show a certain constant relationship to portal or hepatic veins In persistent nonfatal hepatitis there may occur a breakdown of the hexagonal pattern into pseudolobules (shriveled acini) with localization of the lesion in Zone 3 but this pseudolobulation is *not* generalized In the other form of chronic hepatitis postnecrotic or coarse nodular cirrhosis the hexagonal pattern stays unaltered in the larger nodes

The histogenesis of both lesions can be explained on the basis of the acinar concept In a case of coarse nodular (postnecrotic) cirrhosis (Figure 15) only the circulatory periphery of the normal acinar agglomerate shown in Figure 6 has been damaged while the outskirts of its complex and simple acini have not suffered yet This is the case in the liver of an 82 year old man who had suffered from jaundice for 5 weeks before death His slightly smaller but not nodular liver showed subacute necrosis Note that the surviving node of parenchyma (Figure 16) is supplied by



Figure 15 *Scheme of lesions in coarse nodular (postnecrotic) cirrhosis* The distribution of the fibrous bands is outlined in the normal human acinar agglomerate shown in Figure 6 There is an accumulation of fibrous tissue at the circulatory periphery of the agglomerate and along its larger and smaller afferent pathways The coherence of the simple and complex acini within such a parenchymal clump is still maintained and thus also the hexagonal pattern

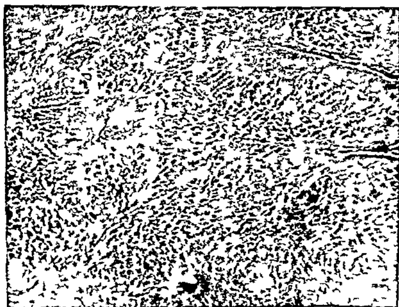


Figure 16 The parenchymal node represents an acinar agglomerate suppl ed by three large vascular branches (right side) Note that the periphery all around the agglomerate is necrotic because of hepatitis The 3 vert r breaks in the tissue are artifact (Human l ver hematoxylin and



FIGURE 17 Schematic outline of lesions in persistent hepatitis coexisting with postnecrotic cirrhosis (See Figure 15) The unity of the parenchymal clump is broken down by fibrous bands which have developed at the periphery of simple and complex acini The acinar agglomerate is subdivided into its component smaller units In each of them the surviving parenchyma is seen centered around the supply lines The efferent hepatic veins lie as ever *between* the acinar units although segregated here in fibrous tissue

three large vascular branches It represents an acinar agglomerate within which the normal relationship between portal and hepatic veins is maintained The necrosis surrounding this agglomerate has halted at its periphery

In the less frequent granular form of posthepatitic cirrhosis which some authors ascribe to a persistent nonfatal hepatitis repeated viral attacks have injured the outer zones of both larger and smaller units severing their coherence (Figure 17) There occurs a breakdown of the hexagonal pattern into single and complex acini which when sufficiently generalized will block the outflow of portal blood into the terminal hepatic veins and cause clinical manifestations of portal hypertension

Discussion

Recent research on the intrahepatic distribution of the hepatic vessels in the gross has shown the dependence of certain parts of the liver upon the supply by certain vascular branches Indeed the same relationship between cells and their nutrient vessels holds for all parts of our body

and I do not think that its validity stops at the microscopic threshold of the liver It is hard to understand why in the liver the organization of its cells into structural units should depend on the efferent vasculature One can find[6 7] the argument that because of the intensive metabolic exchange between liver cells and blood the liver is rather of endocrine than of exocrine structure and therefore its units are oriented around a vein This statement however sounds more like an excuse than a factual observation It is the wish to cling to the traditional that makes such writers miss the point of structural comparison between liver and endocrine organs The similarity consists in the wide sinusoidal capillaries between cell cords (plates) and not in efferent central veins to which cords radiate Such veins do not exist in the endocrines

The liver from an embryological viewpoint should be conceived as developed along the biliary and vascular tree that has grown into the septum transversum to increase the metabolic activity of the gut If we view its branches spread out as in a plant we have flattened for drying a large parenchymal web would result of microscopic thickness equal to the length of a radial sinusoid This web is then seen subdivided on one side by a regularly branched pattern of afferent vessels on its other side there is the interdigitating pattern of its efferent hepatic veins In addition there are on the afferent side the efferent channels of the biliary and lymphatic systems Circulatory activity in such a web shifts from one large afferent area to the other and the waves of blood flow caused by heart beat and respiration finally reach the terminal afferent branches The latter become active as shown in a transilluminated edge of a young rat's liver made in Dr Knisely's laboratory From the axial terminal portal branches the circulatory activity spreads at once into the bilateral sinusoidal network which supplies the area of parenchyma extending on both sides of the vessel into two adjacent hexagonal fields marked by these two central veins This area irrigated by its axial vessel represents a simple acinus the hepatic structural unit in function

ACKNOWLEDGMENTS

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2

*Multiple Parameters Needed to Adequately Describe the Status of the Hepatic Circulation**

RALPH W BRAUER PH D

(San Francisco California)

When I was asked to speak to you originally it was suggested that I talk on some aspects of the physiology of the normal liver that might have a bearing on this subject As Dr Best pointed out this always involves considerable soul searching because the transition from normal to pathological physiology in the liver is a rather painful subject

However there is one region that it seems to me offers obvious implications for the sort of thing that you—and that we—might be concerned with namely the area of the hepatic circulation and therefore I have chosen to talk about this

There is one other reason for picking that topic today It is almost a decade (minus two months I believe) since Sir Harold Himsworth presented in Boston a brilliant series of lectures in which he dramatically called attention to what he termed the circulatory factor in liver disease[1]

On rereading his book I find that his views can be summed up in two statements as far as this particular topic is concerned The first is that there is a sequence of events in the liver starting if you will from some liver injury which in turn causes a derangement of the hepatic circulation this in turn causes more liver injury and in this fashion you get the setting up of a vicious circle of liver disease

The second statement resulted from a good deal of soul searching rather obviously on Dr Himsworth's part and wound up in a brief summary which says essentially that the status of the blood supply to the hepatic parenchyma barring some very special deviations can be adequately described by measuring the total blood flow through the hepatic parenchyma

Today in taking stock of what has happened during the intervening

* The opinions and assertions contained in this report are those of the writer and are not to be construed as official or as reflecting the views of the Navy Department or of the naval service at large (Article 1252 U S Navy Regulations, 1948)

years I will re examine statements and see how they would sound in 1956 in fact this reformulation is a summary of what I would like to say to you

The first statement still holds I think there is reason to suspect that a vicious circle of liver disease does exist which does encompass a sequence of injury circulatory impairment more injury In contrast to Dr Himsworth however I have no illusions that anyone has actually demonstrated that such a circle exists We take it as an article of faith

The reason this has never been demonstrated is encompassed in what has happened to alter the second statement In my opinion there is now ample evidence for saying that the status of the hepatic circulation cannot possibly be understood if the only measurement we have is a measurement of total hepatic blood flow it behooves us to develop methods that are far more sophisticated for describing qualitative aspects of the hepatic circulation and the clumsy and elaborate title I chose for this talk is an expression of the sort of thing I think might be needed in order to approach the subject

I would like to briefly document my claim and then point out some lines of experimentation that I think might allow us in the next decade perhaps to come up with the measurements that we need in the normal and possibly in the pathological liver

The first item of documentation is familiar to most of us namely the simple fact that in a number of conditions the liver finds itself with more blood than it knows what to do with and we happily call this congestion of the liver but if we look at such states from the point of view of circulatory dynamics two things stand out One is the gross derangement of the circulatory patterns which is expressed for instance in such things as the inversion of the lobular pattern which you can see in congested livers The other might be illustrated by Figure 1 an old slide of mine showing the effect of histamine infusion into the portal vein of the dog This procedure will give intense congestion and the figure shows that a sharp increase in lymph flow results when one compares sodium chloride infusion to the histamine infusion the increased lymph flow furthermore is reversible and can be elicited at will by resumption of the histamine infusion

I could equally well have chosen illustrations from Dr Whipple s work in which the same type of vascular pattern was produced by merely placing a ring around the vena cava above the liver I could also have shown the same behavior in quite a number of other conditions all of which have in common congestive filling of the hepatic blood vessels Severe derangement of the fluid matrix of the liver is a common feature in all of these experiments.

This pattern of increased [illegible] volume in the liver of hepatic con

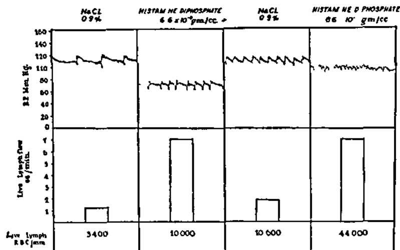


FIGURE 1 Effect of intraportal infusion of histamine or of physiological saline at the same volume rate on liver lymph flow and erythrocyte count in the dog Alternating periods of control and of histamine infusion

gestion is very clearly something that needs to be taken into account the parameter we need to describe such changes obviously is an estimate of hepatic blood volume As far as I know that has never been made in the living animal

The second type of circulatory complication that I would like to call to your attention actually was first described many years ago by our friends at the Mayo Clinic[3] Dr Bollman Dr Mann and others presented some excellent experiments in which they simultaneously measured by stromuhr methods blood flow through the portal vein and the hepatic artery

Regardless of the value attached to numerical results of thermostromuhr measurements in large vessels the results conclusively showed that you could get wide fluctuations in the ratio of hepatic arterial to portal venous flow in the absence of any obvious reasons why such fluctuations should exist

This recently has come to the fore as a result of a critical evaluation of the very beautiful method for estimating total hepatic blood flow developed by Bradley and his co workers Werner and Horvath[4] reported with obvious anguish that they could not get exactly the same measurement twice in fact they got quite wide fluctuations in the concentrations of bromsulphalein in the hepatic veins even over short periods of time Those who are familiar with the method will immediately recognize that it will give the indication of rapid and gross changes in estimated



FIGURE 2 Examples of diffuse and of restricted blood flow distribution in the liver of the rat (From Prichard and Daniel[5])

hepatic blood flow Werner and Horvath concluded that the measurement only gave a mean hepatic blood flow also however and more important for our purpose they did get indications of rapid fluctuations of hepatic blood flow

The most dramatic illustration is even more recent, and comes out of the work of my friends at the Nuffield Institute who by means of cineangiography tried to study the course of blood through the liver and found at least two grossly different distribution patterns (Figure 2) Both pictures in this figure were taken in the same animal, at about the same interval after the injection of the material In one case you get a very nice uniform distribution of the contrast mass throughout the liver and in the other case you get a far more restricted distribution

These pictures represent maximal distributions of the injection mass through the tissue From such observations Dr Prichard and Dr Daniel enunciated the conclusion that there were situations in which the hepatic circulation of the rat would switch from a diffuse circulation of the type you see on the top to a more restricted state which you see on the bottom and parenthetically they could not quite tell why this should happen when it did

Clearly this circulatory change would require rather dramatic methods of measurement What would happen to the liver while this measurement was being attempted I believe I can leave to your imagination

The third piece of evidence indicating that blood flow through the liver is not just a nice quiet steady affair comes out of observations of groups like Wakim and Mann at the Mayo Clinic and Knisely at South

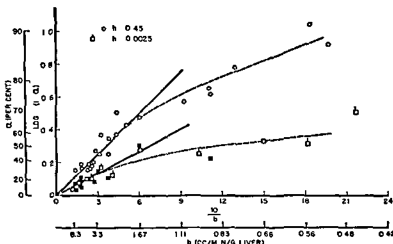


FIGURE 3 Relationship between perfusion rate (b) and extraction efficiency (α) for $CrPO_4$ radiocolloid in the isolated rat liver preparation when whole blood (h = 0 45) or blood plasma (h < 0.0025) at 1 or at 3 atm po is employed

Carolina who in transillumination experiments of the liver demonstrated that the circulation through any given microscopic field is not stationary but that there is a great deal of what they have called vasomotion going on

If you wish to read that literature you will find it rather entertaining because no two authors really agreed on precisely what they saw except that most workers saw that things can change rapidly and that the changes might be both regional and microscopic in scale So we do have evidence then of microscopic as well macroscopic changes in hepatic blood flow distribution

The next line of approach comes out of work which we have done with the isolated rat liver A radioactive colloid (chromic phosphate) was injected and the effect of different rates of perfusion on the efficiency with which this material was extracted was measured One can very easily show that the material behaves rather simply and follows first order kinetics[6, 7] Under those conditions there ought to be a simple relation between the logarithm of the extraction efficiency and what one might call the transit time —the time that a given particle of blood stays in the liver[7]

The results of such experiments are plotted in Figure 3 on an odd system of co ordinates in which if the vascular system of the liver did not change the values ought to follow straight lines In fact as you see at high perfusion rates the data do follow the theoretical lines

Unfortunately as you come to lower perfusion rates there is a sharp

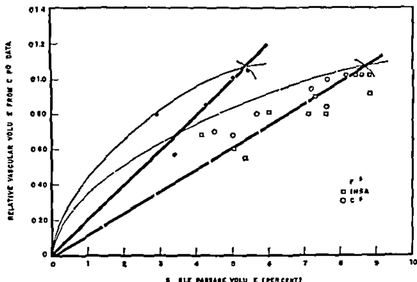


FIGURE 4 Relationship between relative vascular volume from $CrPO_4$ measurements[7 9] and single passage erythrocyte (Fe[9]) and plasma (IHSA and Cr^{51}) volumes[9] in the undisturbed isolated rat liver perfused at different perfusion pressures.

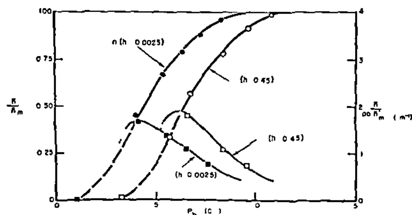


FIGURE 5 Relationship between perfu n pre ure (P_b) and the proportion of all possible channels of th hepatic vasculature actually perfused at any given pressure $\left(\frac{\bar{n}}{n_{max}}\right)$ in the isolated rat liver when either h le blood ($h = 0.45$) or blood plasma ($h < 0.00$) is used as the perfusate[9] Also included is the rate of change of $\bar{n}$ with P_b ($\bar{n}^1$)
n with P_b (n^1)

deviation between what you would predict and what you find The deviation is in such a direction that there is less increase in extraction efficiency than one would expect and this means that either the blood stays less long in the liver or that something happened to the Kupffer cells All things considered it seems most likely that these deviations actually reflect a relative decrease in the transit times or in other words in the volume of the vascular tree with low perfusion pressures

There are two possibilities as to how this could happen Either the vessels could get smaller in diameter so that the total hepatic blood volume could decrease or else there could be a decrease in the number of vessels actually perfused

There is a mathematical relation which allows you to separate the two cases since the reaction rate depends on the mean surface to volume ratio of the vessels if vessel diameters changed — as in the first alternative — the reaction should proceed a little faster while in the second the surface to volume ratio remains constant and the reaction rate should too

Since we have direct measurements of the blood volume in our case[8] we can calculate the relation between transit time and extraction efficiency for these two alternatives (Figure 4) This figure shows straight lines which represent the assumption that the number of vessels perfused is changed whereas the dimensions are not The parabolas on the other hand are based on the assumption that the diameter of the vessels has changed while their number has remained constant It is obvious I think that for both sets of data shown in Figure 4 the one that fits far more adequately is the former assumption that it is the number of open vessels which changes

If you take these results and put them together with other data such as those from bile flow or from flow resistance[9] studies you can construct for the isolated rat liver at least a diagram which correlates the perfusion pressure with the percentage of vessels that are perfused The main reason for showing this otherwise rather elaborate figure (Figure 5) is to indicate that if normal blood with a hematocrit of about 0 45 is used the region in which the number of open channels changes most rapidly comes very close to the region of normal portal pressures which is around 10 to 12 cm in the rat[9]

Thus we find in these experiments that we have evidence of a rather critical balance of the hepatic circulation and at the same time I think we have the makings of a useful method for approaching some of the requisite measurements

In summing up we see that there are at least three lines of evidence which independently indicate that the hepatic circulation is not a stationary thing and that it can undergo rather drastic changes in distribution of blood flow to the parenchyma

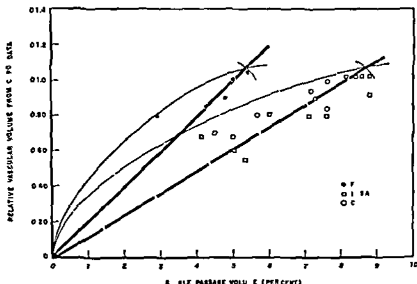


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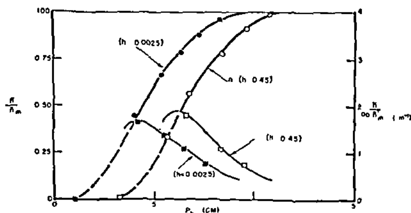


FIGURE 5 Relationship bet veen perfusion pressu e (P) and the pro portion of all possible channel of the h patic asculature actually perfused at any given pressure $\left(\frac{\bar{n}}{n_{max}}\right)$ in the isolated rat l er when either whole blood ($h = 0.45$) or blood plasma ($h \leq 0.00$) s u ed as the perfusate[9] Also included is the rate of change of $\bar{n}$ with P_b ($\bar{n}^1$)
n with P_b (n^1)

attained by changes in perfusion pressure What you are seeing here is a drastic decrease in the total number of vessels through which the blood can flow as well as a decrease in vessel diameter

So here we have three situations each of which at least potentially can occur in clinical experience and if you add to this the experience of those who have been treating hepatitis in man — and I take it this includes most of you at one time or another — that allowing a patient to get up and walk around reasonably early during the course of his hepatitis is apt to have disastrous consequences for his recovery then you can see that here may well be a very important tie up between blood flow distribution measurements and clinical reality

I suppose all this is very good theoretically but if we are going to tie it up with actual developments in the liver and with the course of liver disease or with our understanding of liver physiology we must devise means of providing clinical measurements and clinical observation in the reasonably intact patient — not the physiologist s intact patient who has a bile fistula and three catheters in three places but in the unopened patient who is likely to survive

For this I have three suggestions The first I think is a rather obvious one It seems to me that the cineangiographic techniques with the recent development in intralienal portography possibly would lend themselves effectively to getting a piece of the information we need if the procedures are not too hazardous

For experimental and practical purposes the technique that Dr Hoffbauer[1] and others used of implanting a catheter in some tributary of the portal tree might conceivably make the same thing possible with a little less risk At any rate such observations coupled with the measurement of estimated hepatic blood flow I think would considerably amplify our understanding of circulatory changes However as of now cineangiography is a bit specialized because it will not show anything but the gross blood flow distribution changes

As a more powerful though more laborious tool I would like to suggest a second approach and that is essentially an adaptation of a type of apparatus which I have used for the isolated liver It is the thing that gave the title to this talk namely that in order to understand what is going on with the blood supply to the liver we think we may be quite well off if we can get at any one time three parameters If we can get measurements for instance of total blood flow through the liver of hepatic blood volume and some measurement which is sensitive to vessel diameter changes (and this might be resistance or chromic phosphate extraction or one of a number of other things) then between these we can construct a set of normal values by which I think we can gauge any changes in blood flow distribution through the liver

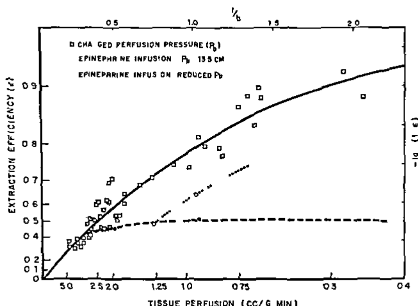


FIGURE 6 Relationship between perfusion rate and $CrPO_4$ extraction efficiency for the isolated rat liver preparation when blood flow is changed either by altering perfusion pressure or by continuous epinephrine hydrochloride infusion

That these phenomena can be of real clinical importance can be indicated by just a few observations One is the very nice series of observations by Dr Olerud at Uppsala [10] who has shown that if the intra abdominal pressure is increased there is precisely the same difference between diffuse and restricted circulation seen in the observations of Prichard and Daniel and a great deal of injury is also seen under those conditions

Again I might remind you of Dr Bradley s observations [11] He showed a number of years ago that the change from the reclining to the upright position is accompanied by a sharp change in portal blood flow and in splanchnic blood flow but he did not point out (although his data showed it) that this change also is associated with a sharp decrease in bromsulphalein extraction efficiency a decrease that I find it very difficult to account for other than to assume some sort of a transition from unrestricted to restricted flow

In the third place I will show one other slide on the effect of epinephrine on the liver (Figure 6) If you will recall Figure 3 which correlated the extraction deficiency with the tissue perfusion you will recognize the normal liver relations and the effect of epinephrine If perfusion rate is lowered by epinephrine infusion you see that the chromic phosphate extraction fails to increase as it would if the same perfusion rates were

The technique for instance would be to make simultaneous injections of radioactive and nonradioactive bromsulphalein into the portal vein and into the general circulation respectively (if you cannot get at the hepatic artery) and then estimate the excretion ratios to get some estimate of the ratio of blood flow if you know the excretion ratio directly from the surgical preparation Clearly this is not a practical method for the clinic at the moment However I think all three of my suggestions are potentially practicable methods and all are badly needed if the physiologist is ever going to be able to make a useful contribution to a meeting of this type in terms of an understanding of the circulatory changes that proceed and accompany liver injury

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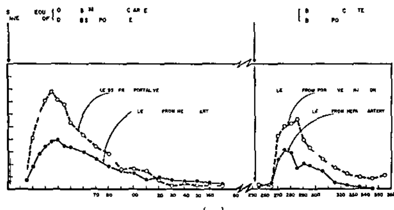


FIGURE 7 Differences in biliary bromsulphalein excretions in the dog due to route of administration

Finally lest you think I am not aware of this problem may I remind you that a third problem we have and that we love to neglect because it is execrably hard to get at is the problem of estimating hepatic arterial blood flow How do we get it? I have one suggestion to make on that Figure 7 might illustrate one approach It comes from the fact that about two years ago Dr Maegraith at Liverpool wrote me a letter and said You know all this talk you have been having about bromsulphalein extractions is very nice but I don t think it makes any sense because when Dr Andrews and I inject this stuff into the portal vein I get entirely different phenomena from what I get when I inject it into the hepatic artery [13]

This bothered me because I have been arguing that these extractions occurred in the sinusoidal bed and some have said that they should occur in the bile duct regions and since at about the same time the peribiliary arterial plexus had been described these things seemed to tie together too well to be ignored so I did the following experiment

I took some radioactive bromsulphalein and some nonradioactive bromsulphalein I injected the first into the hepatic artery of the dog and the nonradioactive bromsulphalein into the portal vein simultaneously and then I collected bile To my intense comfort I found that under those conditions the material that goes into the portal vein is excreted far more rapidly into the bile than the material that goes into the hepatic artery Reversing the order of injection gives the same results Here then is a first indication that we may be able to construe and in fact an experimental preparation from which we can construe a method of estimating hepatic arterial blood flow by looking at the handling of a substance like bromsulphalein

3

Pathologic Physiology of Hepatitis

LEON SCHIFF M D PH D

(Cincinnati Ohio)

The histologic changes of viral hepatitis are the result of rapidly in duced damage and necrosis of liver cells cellular inflammatory reaction and cell regeneration occurring side by side [1] Physiologic changes result from regurgitation of bile into the blood caused by cholangiolar injury and intrahepatic obstruction With necrosis of liver cells there results impairment of numerous metabolic functions of the liver and liberation of cell constituents including iron, enzymes and vitamins Since neither knowledge nor the time allotted permits an adequate discussion of the pathologic physiology of hepatitis I shall limit my remarks to some ob servations dealing with serum bilirubin serum bile acids serum alkaline phosphatase plasma ammonia serum iron and plasma prothrombin

SERUM BILIRUBIN

In the past it was often stated that the renal threshold for direct bilirubin was 2 mg per cent The threshold has been found to be quite variable in hepatitis not only in different individuals but at different stages of the disease [2, 3] In the preicteric phase of hepatitis or in anicteric hepatitis the one minute bilirubin is significantly elevated when the total bilirubin is within normal limits and this elevation is usually accompanied by biliru binuria [4, 5] During the icteric phase of hepatitis the bilirubin ratio $\left(\frac{1}{T} \times 100\right)$ is greater than 50 per cent in about three fourths of the cases (in contrast with more than four fifths of the cases of biliary obstruction due to tumor) [3] In the subsiding stages of hepatitis bilirubin disappears from the urine at a considerably higher level (0 8 to 1 2) than that at which it appears in the urine in the preicteric stage It is possible that an increased bile salt concentration in the urine during the early phases of viral hepatitis and a diminished content during the later ones may account for these variations in the renal threshold but this is not known.

At times retention jaundice may be observed in the subsiding or con valescent phases of infectious hepatitis and may persist for months or longer When it persists indefinitely the possibility of antecedent con stitutional dysfunction must be considered [5, 6] It has been suggested that

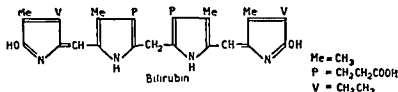


FIGURE 1 From Gray C H *The Bile Pigment* London Methuen 1953

conjugated bilirubin [13] Schmid cites a case reported by Rosenthal *et al* [16] in which a child with congenital nonhemolytic jaundice with 30 mg per cent of free bilirubin in the serum had no bilirubin in the urine

Just what happens to the conjugating mechanism of bilirubin in hepatitis remains to be determined in the light of these newer developments and those to follow The lesser incidence of bilirubin ratios of over 50 per cent in cases of hepatitis as compared with biliary obstruction due to tumor (as pointed out by Watson) suggests impairment of this mechanism in some cases of hepatitis It seems reasonable to assume impairment of this mechanism in posthepatitic hyperbilirubinemia and constitutional hepatic dysfunction

Hemolytic anemia may rarely be associated with viral hepatitis I have seen such an association but three times In two of these cases otherwise uncomplicated the anemia and hepatitis disappeared simultaneously Figure 2 depicts some data obtained from the third patient (M L K J H #483318) with homologous serum hepatitis and acute hemolytic anemia The patient a white female of 56 years had undergone subtotal gastrectomy for Hodgkin s disease of the stomach eight years before without subsequent evidence of recurrence Between February 29th and March 11th she was given 3 units of packed cells prior to resection of a carcinoma of the splenic flexure on March 13 1956 Because of adherence of the tumor to the spleen splenectomy was also performed Jaundice appeared on April 27th The prompt (one minute) serum bilirubin concentration on May 3 1956 was 18 mg per cent and the total bilirubin 30 mg per cent The flocculation tests were positive and bilirubinuria was present The Coomb s test was negative She was placed on steroid therapy and given three more units of packed red cells as indicated in Figure 2 which also depicts the decrease in reticulocytes and serum bilirubin with concomitant rise in the red blood cell count The occurrence of hemolytic anemia in spite of splenectomy is noteworthy

A patient with viral hepatitis and jaundice treated with hydrocortisone (R R C G H #336754) has just been studied with Dr Yoichi Oikawa at the Cincinnati General Hospital Much to our surprise the stools remained acholic during the sharp drop in serum bilirubin and the quantity of bilirubin in the urine decreased This is in keeping with an observation

constitutional hepatic dysfunction may be a sequela of an anicteric form of infectious hepatitis [7,8] and not a disease *sui generis* The familial incidence of this disorder may be explained by several members of a family having been stricken with hepatitis at about the same time (or during the same epidemic) Kalk [9] has reported in cases of posthepatitic hyper bilirubinemia the occurrence of brown pigment in liver cells similar to that observed in the Dubin-Johnson syndrome but this awaits confirmation He believes the pigment to be a particular form of masked iron bound to protein or lipoid bodies in such a way as not to give a typical iron reaction

The hyperbilirubinemia of infectious hepatitis is predominantly of the prompt reacting type and may be explained by a combination of factors operating in varying degrees These include cholangiolar injury producing increased permeability and leakage of bile into adjacent lymph spaces and blood,[5] and intrahepatic obstruction due to edema swelling of liver cells disruption of the liver cell columns with their associated intercellular bile canaliculi and bile thrombi Watson has postulated cholangiolar injury as the physiologic basis of long standing hepatitis with remarkably good liver cell function — so called cholangiolitic hepatitis

In the past three years [10,11] but more particularly in the past year [1,13] notable advances have been made in the elucidation of the change which bilirubin undergoes in its passage through the liver cells since attention was first directed to this phenomenon by van den Bergh and Muller forty years ago [14] The difference between the indirect and direct reacting bilirubin seems at last to have been clarified It has been shown independently by Billing and Lathe [1] in England and by Rudi Schmid [13] in this country that bilirubin is conjugated in the liver with glucuronic acid * Schmid believes that the glycosidic linkage probably occurs at the hydroxyl groups on the two outside pyrrol rings of the bilirubin molecule and is an ether [13] (see Figure 1) while Billing and Lathe feel that the linkage occurs through the proprionic acid groups and represents an ester As is true of other substances that are conjugated in the liver with glucuronic acid the glucuronide is far more soluble in water than the parent substance and is consequently more readily excreted in the urine [15]

Most of the direct reacting bilirubin appears to be present as a di glucuronide and smaller amounts as a monoglucuronide [13] In bile most or all of the bilirubin is excreted as a water soluble glucuronide In regurgitation jaundice conjugated bilirubin gains access to the blood and thence to the urine resulting in bilirubinuria The kidneys can excrete only

* Since presenting this paper my attention has been called by Barbara Billing to the communication of E. Talofunt of Czechoslovakia (*Nature* 178 311 1956) confirming the diglucuronide composition of the directly reacting pigment obtained from bile

crease is delayed[20] It is not delayed in jaundice due to obstruction of the bile ducts [0]

Using a modification of Josephson s method [1] Sherlock and Walshe[2] reported an increase in the mean of blood cholates in acute hepatitis over the normal of 0 to 3 0 mg per cent obtained by their method but results were variable The values obtained did no significantly diminish in the patients showing the more severe degrees of hepatic damage The level was found to be highest early in the disease falling with recovery A positive correlation existed between the serum bilirubin and blood cholate determinations but there was no correlation with serum alkaline phosphatase total cholesterol or the serum proteins The theoretically expected diminished bile salt production could not be proved The increased concentration was ascribed to interference with bile salt excretion as a result of the intrahepatic changes which characterize hepatitis

Carey [23] [4] has determined the bile acid concentration in human serum from the appropriate ultraviolet absorption maxima after extraction by Josephson s method and hydrolysis He found the mean trihydroxy bile acid (cholic acid) value for 35 healthy subjects to be 1 1 plus or minus 0 4 mg per cent and the dihydroxy bile acids (deoxycholic and chenodeoxycholic acid) to be 0 4 plus or minus 0 mg per cent giving a ratio of 2 3 In jaundiced patients with severe liver damage as in hepatitis the trihydroxy dihydroxy bile acid ratio was completely reversed to less than 1 with an average of 0 71 This reversal was accounted for by the greater than fivefold increase in the dihydroxy acid values the group average being 2 7 mg per cent The average trihydroxy value was however only slightly increased Among 15 patients having extrahepatic biliary obstruction without appreciable hepatocellular damage the concentrations of trihydroxy and dihydroxy bile acids were greatly increased as much as tenfold in some cases while the ratio between the two remained normal

We have used Carey s method for the past six months and have been able to confirm his observations in general Dr Carey points out that the method has seemingly proved useful in the differential analysis of the serum bile acids especially when the concentrations are slightly elevated although it is still in a developmental stage The following curves have been obtained in our laboratory with the assistance of Dr Illa Stern and Eugene R Schiff

Figure 3 shows the curve obtained from a normal subject The ratio of the trihydroxy to the dihydroxy acids is somewhat less than average In Figure 4 is depicted a curve obtained from a patient (C R H H #551148) with a stricture of the common bile duct with very marked pruritus and is in keeping with the type of curve which Carey has usually found in cases of extrahepatic biliary obstruction Figures 5 and 6

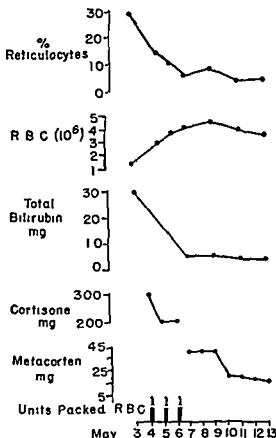


FIGURE Homologous serum hepatitis with hemolytic anemia

reported by Chalmers *et al*[1] in a case of cholangiolitic cirrhosis and raises the question as to the mechanism of the decrease in serum bilirubin following steroid therapy [1] Certainly neither an anti inflammatory effect with resorption of periportal exudate nor a choleretic effect provides an adequate explanation

Serum Bile Acids

The conjugation of bile acids is related to detoxification in general and is accomplished by peptide linkage with either glycine or taurine In parenchymal liver damage the supply of glycine or taurine as well as the enzyme is reduced and is insufficient for the conjugation of endogenous as well as exogenous bile acids[19] In normal persons and in patients with biliary obstruction after intravenous injection of cholate the blood level rapidly returns to normal while in parenchymatous liver disease the de

INFECTIOUS HEPATITIS (C.P, 9845)

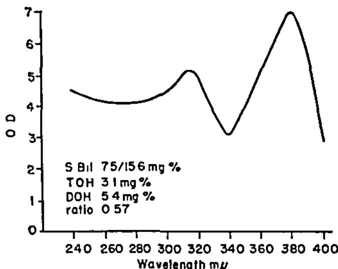


FIGURE 5

show curves obtained from patients with infectious hepatitis and are in keeping with those described by Carey in cases of liver damage Figure 6 represents the values obtained on the second day of jaundice (and illness — there was no recognized preicteric phase) in a former laboratory technician of ours Figure 7 is particularly interesting in that it shows an obstructive type curve in a colored male aged 4 years on the fourth day of jaundice The clinical and laboratory findings in this case were typical of infectious hepatitis except that pruritus was so prominent as to clinically suggest obstructive jaundice Needle biopsy of the liver revealed the classical changes of viral hepatitis with a minimum of bile stasis This case experience suggests that estimation of serum bile acids will prove more of a physiologic than a diagnostic tool

Figure 8 (taken from Siperstein and Chaikoff [5]) shows the probable pathways of the formation of bile acids from cholesterol under normal conditions From the results obtained by Carey and in fewer cases studied in the Gastric Laboratory it appears that in hepatitis a block occurs in the hydroxylating enzymes thus interfering with the formation of trihydroxy from dihydroxy bile acids

SERUM ALKALINE PHOSPHATASE

The mechanism underlying the changes in the serum alkaline phosphatase activity in hepatic and biliary tract disease has yet to be clarified [6]

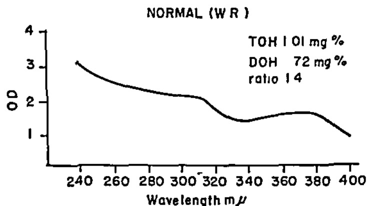


FIGURE 3

STRICTURE OF THE COMMON BILE DUCT (M C)

S Bil 11/2 1 mg %
TOH 9 9 mg %
DOH 3 2 mg %
ratio 3 1
S A P 74 8 U

OD

11
10
9
8
7
6
5
4
3
2
1
0

240 260 280 300 320 340 360 380 400

Wavelength mμ

FIGURE 4

blood stream The absence of a significant increase in extensive hepatocellular damage would be explained by failure of production

Figure 9 is based on data previously published[27] and represents evidence of diminished production of the enzyme when the liver is severely damaged by cholangitis secondary to biliary obstruction due to common duct stone When the serum bilirubin was at its highest level (36 mg per cent) — and extensive damage to the liver was verified by a needle specimen of the liver — the serum alkaline phosphatase was only 8 Bodansky units As the cholangitis lessened (as revealed in a second biopsy specimen) and as liver damage and biliary obstruction decreased the alkaline phosphatase rose to a maximum of 56 Bodansky units

Plasma Ammonia

Zimmerman *et al* [9] have recently reported frequent increase of the plasma ammonia concentration in cases of viral hepatitis The elevations were noted during the period of greatest intensity of the disease but not during convalescence (Figure 10) The increase appears to be due to impairment of the liver s ability to metabolize ammonia since the factor of bypassing of the liver should play little if any role This is also borne out by the data shown in Figure 11 in which elevation of the blood ammonia concentration followed the intragastric administration of 500 cc of citrated blood to a patient with infectious hepatitis in contrast with the results obtained in a group of eight normal control [9]

A high protein diet acting as an increased source of ammonia might therefore be theoretically harmful in hepatitis Clinical observations have been somewhat contradictory in this regard Chalmers *et al*[30] reported shortened duration of jaundice in patients on a high protein diet while Leone and associates[31] reported a longer duration of illness and more frequent complications in patients receiving high protein high carbohydrate and restricted fat diets

Serum Iron

Since Warburg and Krebs s original report[3] of an increase in the blood iron in a jaundiced patient, numerous studies have appeared on the serum iron in hepatobiliary disease[33–41] The serum iron is particularly increased in hepatitis (Figure 12) and reaches its highest value in the second and third weeks of the disease[35] attaining a mean value of 240 to 300 gamma per cent as compared with a mean value of 100 to 143 gamma per cent in normals[41] The maximal serum iron level follows the maximal increase in serum bilirubin[35 37 41] with the values returning to normal after the eighth week[4] Hemmeler[33 34] has stated that the serum iron may be low or normal when jaundice appears but it soon increases The increase in serum iron is thought to be due to the liberation of iron from

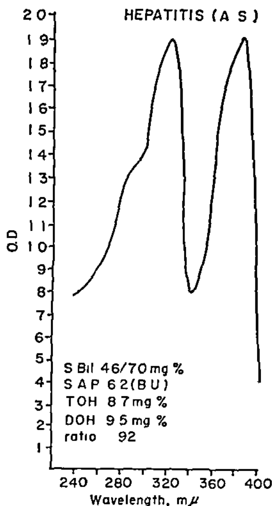


Figure 6

It has been suggested that the increase in serum phosphatase activity which has been observed in jaundice of obstructive or hepatocellular origin may represent a retention phenomenon dependent upon extrahepatic or intra hepatic obstruction to the flow of bile On the other hand Cantarow and Trumper point out that some observers believe the alkaline phosphatase which accumulates in the blood in biliary obstruction or hepatocellular disease originates in the liver cells or bile duct epithelium and instead of being excreted in the bile enters the blood stream via lymphatics or portal sinusoids According to this view the phosphatase occurring normally in bile is not that originating in the skeleton and present in the

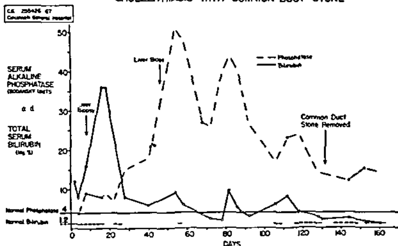


FIGURE 9 A monograph Schiff L., *Clinical Approach to Jaundice* Springfield Ill Charles C Thomas.

Ammonia Levels During Course of Viral Hepatitis

Plasma NH_3 γ/ml

3.0 2.5 2.0 1.5 1.0 0.5 0

Weeks 1 2 3 4 5 6 7

FIGURE 10 Scattergram showing ammonia level during successive weeks in the hospital in 35 patients with viral hepatitis. (From Zimmerman Korn and Weinstein *A I M S* 1956)

INFECTIOUS HEPATITIS (R H 268775)

8-29 56
S Bil 20/42 mg /
S A P 58 B U
TOH 38 mg /
DOH 33 mg /
ratio 12

8 14 56
S Bil 92/148 mg /
SAP 70 BU
TOH 67 mg /
DOH 535 mg /
ratio 125

O D

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

260 300 340 380

260 300 340 380

Wavelength, m μ

FIGURE 7

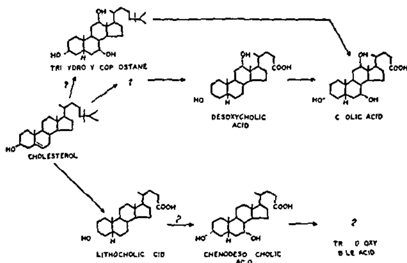


FIGURE 8 Probable path ays of bile acid synthe is (Frcm S perstein a d Chaikoff Conversic n of cholesterol tc bile acids *Federation Proc* 1955)

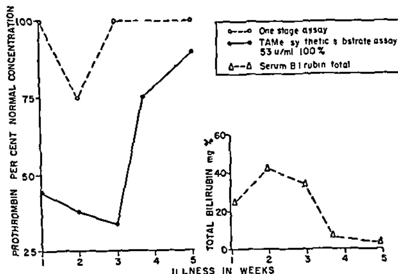


FIGURE 13 The one stage & synthetic substrate assays (TAMe) of plasma prothrombin in a patient with infectious hepatitis

disintegrated liver cells plus a failure of the liver to absorb the iron derived from hemoglobin destruction as suggested by Brochner Mortensen Increased hemolysis may play an additional role Reissmann and Dietrich[4] have reported the appearance of ferritin in the peripheral blood of some patients with infectious hepatitis and attribute its appearance to release from disintegrating liver cells Appearance of ferritin did not parallel increase in serum iron

Plasma Prothrombin

It is well known that plasma prothrombin may diminish in the presence of liver damage and when the damage is severe the decrease may persist in spite of vitamin K therapy Figure 13 kindly given me by Dr Helen Glueck shows a marked decrease in plasma prothrombin as determined by the synthetic substrate (TAMe Assay)[43] in contrast with a slight decrease as measured with the conventional one stage method of Quick This assay in contrast with clotting methods, is relatively unaffected by a deficiency of Factor VII (stable factor) and therefore measures only alterations in prothrombin itself[44] It is seen that the plasma prothrombin rose as the serum bilirubin dropped The patient was a white male of 37 years (L.F.M C R H H #560443) with a rather severe hepatitis Treatment included steroid therapy

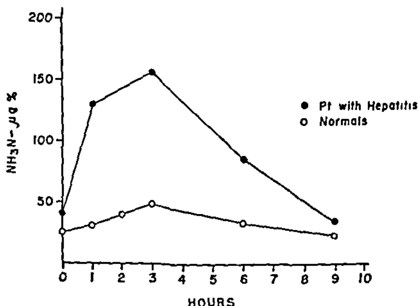


FIGURE 11 The concentration of blood ammonia in a patient with hepatitis compared with that observed in 8 normal patients following blood ingestion

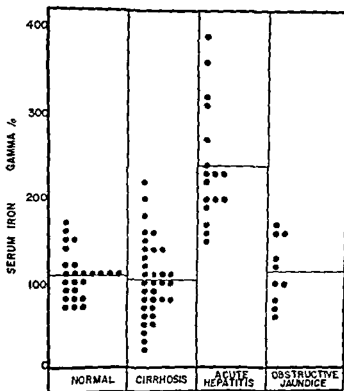


FIGURE 12 Scattergram of the over all results found in 5 healthy subjects and 61 patients with liver disease (From Stone Rumball and Hassett *Ann Int Med* 1955)

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26 Cantarow A and Trumper M *Clinical Biochemistry* Philadelphia Saunders 1955
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28 Zimmerman H J Korn R J and Weinstein, H G Observations on the source of elevated plasma ammonia levels in hepatic insufficiency *Am J M Sc* 231 177 1956
29 Young P C Burnside C R Knowles H C and Schiff L Effect of intragastric administration of whole blood on the blood ammonia blood urea nitrogen and nonprotein nitrogen in patients with liver disease *J Clin Investigation* 35 747 1956
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DESIGNATED DISCUSSION

JESSE L BOLLMAN M D (Rochester Minnesota) It is extremely difficult to discuss such papers as we have heard in a few moments As you notice they are quite diffuse and still fail to cover the entire field That is not surprising because we are here for only three days to cover the field of hepatitis and I am reasonably certain we will not get the field of hepatitis completely covered in three days

I would like to call attention to the fact that both Dr Rappaport and Dr Brauer emphasized the circulatory aspects of the liver Anyone who has injected a mass into the blood vessels of the liver and then corroded the liver cells away is immediately struck with the fact that one hasn t really corroded very much away — that the liver consists of the injection mass and only a relatively small amount of hepatic cells quite different from the impression one gets from histologic section of the liver

In total consideration if we think of the liver as a blood regulating organ we will see that it is functioning to maintain homeostasis in the blood It is not surprising then that the blood reaches each part of the liver and that the liver cells are suspended in extracellular fluid

The plasma filtrate diffuses into this extracellular fluid the hepatic cells then modify the material n the extracellular fluid and can only do one thing that is put it back in changed form or in the form that it is in the extracellular fluid which again is reabsorbed by the blood so that everything goes along fine unless the blood supply is interfered with the hepatic cell injured or the biliary excretory mechanism damaged

Unfortunately in hepatitis all of these things occur so we are going to have to consider hepatitis from three angles — blood bile and hepatic functioning cells

Dr Schiff in the early part of his talk mentioned the fact that bilirubin excreted in the bile is a glycuronate and is water soluble An interesting corollary to that noticed in studies in which we injected radioactive thyroxine or tri iodothyronine into an animal is that fully one third of the thyroxine or tri iodothyronine is excreted in the bile It is concentrated in the bile much more than in the blood and is also excreted in the bile as a glucuronate So we have a suggestion now for another liver function test namely the possible use of some other glucuronate for a liver function test

GENERAL DISCUSSION

CECIL WATSON M D (Minneapolis Minnesota) Dr Schiff referred to the question of the difficulty of conjugation of the free bilirubin with glucuronic acid in hepatitis as compared with neoplastic biliary obstruction I think this difference is remarkably slight As he pointed out, our

own figures show that in some cases of hepatitis there is a relatively low ratio lower than in neoplastic obstruction

It has been intriguing to me that in very severe liver damage even within a few hours of death from hepatic insufficiency the prompt direct reacting bilirubin may comprise a surprisingly high percentage of the total We have seen it in patients with acute diffused necrosis of the liver 24 hours before death the direct reacting bilirubin comprising as high as 70 per cent of the total bilirubin in exactly the same range as in cases of neoplastic obstruction

We can only assume that the conjugating enzyme in the liver must still be active Perhaps it is held there as if in a sponge of necrotic material This is a little hard to believe but we certainly have evidence bearing on that point from other aspects in the same patient We may see the blood urea nitrogen go to levels of over 100 We have observed this on many occasions as have others

Then the matter of the bile acids which Dr Schiff also alluded to I think bears on this point It has been of great interest to me that with Dr Carey s method in patients with severe liver damage again even shortly before death from hepatic insufficiency there may be a considerable elevation of dihydroxy bile acids It would seem that the difference in going from a dihydroxy to a trihydroxy bile acid would be a relatively minor functional event yet it seems to measure the difference between a severely damaged liver and a mildly damaged liver

From a practical standpoint the ratio of the dihydroxy to trihydroxy acids is of some help in diagnosis but I do not think it is of decisive value I would emphasize that there are many cases of relatively mild diffused parenchymal disease of the liver in which there is a perfectly normal ratio —in other words a greater amount of trihydroxy acid So we cannot use this in any decisive separation of shall we say medical and surgical jaundice or parenchymal versus simple mechanical obstructive jaundice

When Dr Carey finds in his now fairly large series that with a marked increase in the dihydroxy acids there is a very much greater likelihood of diffuse liver disease and even more important when this is followed and it is seen that the dihydroxy acids are increasing and the trihydroxy acids going down then this is a matter of real prognostic import

I have been surprised at how sharp he has been on this point In cases where he noted that the dihydroxy acids were steadily rising he said the prognosis would be very poor These proved to be remarkable predictions in relation to some of our patients on the wards as we really didn t expect the outcome to be quite as unfortunate as it was

Charles S Davidson M D (Boston Massachusetts) I would like to add to the interesting comments by Dr Brauer which certainly should be

followed up by extensive clinical investigation I should like to point out however that applying the experiments and studies which he reported directly to the clinic may not always be applicable nor explainable on physiological grounds

A careful clinically controlled study several years ago in Japan pointed out quite clearly I think that exercise during the active phase of hepatitis did not produce a prolonged convalescence nor upset the applecart in other ways

So as yet this correlation is not as good as we would like it to be I am sure he realizes that as well as do the rest of us

own figures show that in some cases of hepatitis there is a relatively low ratio lower than in neoplastic obstruction

It has been intriguing to me that in very severe liver damage even within a few hours of death from hepatic insufficiency the prompt direct reacting bilirubin may comprise a surprisingly high percentage of the total We have seen it in patients with acute diffused necrosis of the liver 24 hours before death the direct reacting bilirubin comprising as high as 70 per cent of the total bilirubin in exactly the same range as in cases of neoplastic obstruction

We can only assume that the conjugating enzyme in the liver must still be active Perhaps it is held there as if in a sponge of necrotic material This is a little hard to believe but we certainly have evidence bearing on that point from other aspects in the same patient We may see the blood urea nitrogen go to levels of over 100 We have observed this on many occasions as have others

Then the matter of the bile acids which Dr Schiff also alluded to I think bears on this point It has been of great interest to me that with Dr Carey s method in patients with severe liver damage again even shortly before death from hepatic insufficiency there may be a considerable elevation of dihydroxy bile acids It would seem that the difference in going from a dihydroxy to a trihydroxy bile acid would be a relatively minor functional event yet it seems to measure the difference between a severely damaged liver and a mildly damaged liver

From a practical standpoint the ratio of the dihydroxy to trihydroxy acids is of some help in diagnosis but I do not think it is of decisive value I would emphasize that there are many cases of relatively mild diffused parenchymal disease of the liver in which there is a perfectly normal ratio —in other words a greater amount of trihydroxy acid So we cannot use this in any decisive separation of shall we say medical and surgical jaundice or parenchymal versus simple mechanical obstructive jaundice

When Dr Carey finds in his now fairly large series that with a marked increase in the dihydroxy acids there is a very much greater likelihood of diffuse liver disease and even more important when this is followed and it is seen that the dihydroxy acids are increasing and the trihydroxy acids going down then this is a matter of real prognostic import

I have been surprised at how sharp he has been on this point In cases where he noted that the dihydroxy acids were steadily rising he said the prognosis would be very poor These proved to be remarkable predictions in relation to some of our patients on the wards as we really didn t expect the outcome to be quite as unfortunate as it was

CHARLES S DAVIDSON M D (Boston Massachusetts) I would like to add to the interesting comments by Dr Brauer which certainly should be

4

Geographic Pathology of Hepatitis

KENNETH R HILL* MD and GERRIT BRAS MD
(Jamaica B W I)

Geographic pathology is the particular study of disease which takes into account the relationship between pathological factors and those of geography We recognize that disease in man is a phenomenon which occurs only if various factors coincide in time and space

According to May[14] these factors may be designated as follows

PATHOLOGICAL FACTORS

1 Causative agent
2 Vectors
3 Intermediate hosts
4 Reservoirs
5 Man

GEOGRAPHICAL FACTORS

1 Physical — Climate latitude humidity temperature
Relief
Soils
Hydrography etc
2 Human or social — Population density
Standards of living
Religious customs
Diet
Sanitation etc
3 Biological — Vegetable life
Animal life
Parasitism
Prevalent diseases
Dominant blood groups

Hepatitis etymologically means an inflammation of the liver this has many wide interpretations We prefer in general that of Payling Wright[25] who defines inflammation as the process by means of which cells and exudate accumulate in the tissues and tend to protect them from further injury However strict conformity to this definition is not always possible particularly when considering the parenchymatous inflammation of say viral diseases here usage is more important than etymology

* Present address Department of Pathology The Royal Free Hospital School of Medicine Grays Inn Road London W C 1 England

to culture the virus of infectious hepatitis on Detroit–6 strain of epithelial cells.

Virus A can be passaged in the feces or urine and is probably water borne it can withstand heating for 56 C. for 30 minutes or freezing −10 to −20 C. from 1 to 1½ years Virus B is found in blood plasma or serum either liquid or desiccated it survives heating to 60 C for 1 hour and remains active at a temperature of −10° to − 0° C. for 4½ years and in a desiccated state at room temperature for one year [73]

Findlay [9] in a study of morbidity rates in West Africa found the incidence of infectious hepatitis higher in visiting troops than in local ones he suggested that this was due to different strains of the virus Although this is theoretically possible it has not yet been verified or disproved

Vectors and Intermediate hosts None are known

Reservoirs Virus A is found in the blood and alimentary tract of adult humans only for a short time but it has been found in the feces of two children for periods of 5 and 16 months respectively [3] Virus B has been found in the blood of an adult after 5 years but has never been shown to be infectious by the intestinal oral route

Much evidence suggests that virus A is propagated by personal contact through the intestinal oral route and that soils water and milk can be contaminated [63, 3] Virus B is transmitted by blood transfusion and parenteral injections or inoculations which incorporate the use of infected blood constituents

Man Both viruses produce similar liver lesions in man and the liver cells are attacked to a varying degree The result of this damage and the subsequent inflammatory reaction controls the morbidity or mortality of the condition Man appears to be the only animal susceptible to these viruses although the virus has been propagated on embryonated eggs [38, 40]

The hepatitides of the animal kingdom such as avian infectious hepatitis [40] viral hepatitis in ducklings [4, 24] canine hepatitis [19] the hepatitis of mice [3] viral hepatitis in horses and Rift Valley fever virus [73] appear not to be related to the viral hepatitides of man

Geographical Factors

Infectious hepatitis appears to be world wide and the incidence appears to rise and fall regardless of terrain or climate However there seems to be a higher incidence in certain areas such as the Mediterranean littoral and Germany where the history of hepatitis epidemics goes back 200 years [21] So much so does this appear to be the case — as if these areas had been seeded by the virus — that in both World Wars the in-

It would be impossible in the short time at our disposal to deal with *all* the hepatitides in relation to geographical factors. However we have been helped by a perusal of the program of this international symposium, *Hepatitis Frontiers* we find that "hepatitis," by inference is that caused by viral agents, and thus we propose to concentrate on this aspect of the disease and add one other which may be of interest.

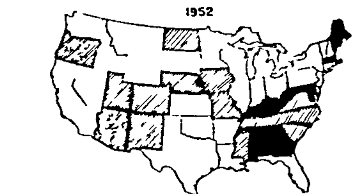
HEPATITIS	ETIOLOGY
Viral	Fetal ( )
	Infectious
	Serum
	Yellow Fever
	Infectious mononucleosis, etc.
Bacterial	Tuberculosis leprosy
	Salmonellosis enteric
	Brucellosis
Spirochetal	Leptospirosis
	Syphilis
Parasites	Malarial
	Schistosomiasis
	Amebiasis
	Flukes, etc.
Toxic	Hepatotoxic poisons, e.g., arsenic, phosphorus, chloroform, etc.
	Septicemia, etc.
	Venous occlusive disease

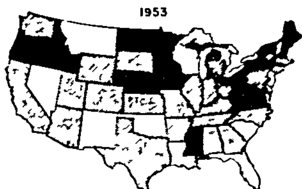
INFECTIOUS HEPATITIS AND SERUM HEPATITIS
Pathological Factors

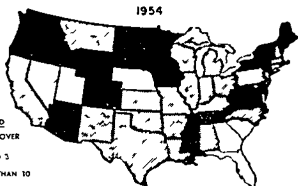
Causative Agent. It is now generally recognized that "infectious hepatitis" differs from "serum hepatitis," in that it is caused by a different virus.

It may be profitable at this stage to discuss the differences between the two viruses, although no doubt other speakers in this symposium will dwell on them in detail.

Briefly from observations in naturally occurring and experimental infection in man (for no satisfactory experimental animal has yet been found) the incubation period of infectious hepatitis is 15 to 40 days and of serum hepatitis, 60 to 160 days.[73] Recent reports by Pollard and Diserens[49] have shown that the viruses of both infectious hepatitis (virus A or IH) and serum hepatitis (virus B or SH) have been propagated on embryonated eggs, and they have shown that the two viruses are antigenically distinct, that virus A is homotypic and that gamma-globulin can neutralize virus A but not virus B. Rightsel *et al.*[55] have recently claimed







LEGEND
35 & OVER
10 TO 3
LESS THAN 10

FIGURE Reported cases of viral hepatitis by state per 100,000 population (From Sherman and Eichenwald *Ann Int Med*)

cidence of infectious hepatitis in invading troops was much higher in these areas than elsewhere [48]

Serum hepatitis is also world wide although to some extent it is found only where modern medical techniques such as blood transfusion services are established It must not be forgotten however that during World War II many thousands of British and American troops inoculated with yellow fever vaccine made from icterogenic human serum contracted this disease (approximately 20 per cent of the American troops so inoculated in 1942) [47]

In general the distribution and spread of the disease are at present inexplicable but it is of interest to note that in the United States states in the southeast which experienced a high prevalence in 195- (Figure 1) saw a decline in the incidence in the following year but a spread to the periphery By 1954 this was further accentuated [57] The explanation may be in the development of immunity within the different populaces but this cannot be the whole answer

Physical Factors In temperate zones infectious hepatitis appears more prevalent in the autumn and winter but summer epidemics also occur In tropical zones the disease may occur at any time of the year

In the United States the seasonal distribution is indicated in Figure 2 [57] Havens [6] pointed out as long ago as 1946 that the prevalence was in the late fall and early winter There have been similar findings in other parts of the world and Figure 3 [57] compares the incidence in Scandinavia with that in the United States

It has been noticed that although the morbidity fluctuation of what is termed viral hepatitis rises and falls with the season the mortality rate remains constant This suggests possibly that the reported cases are in the main infectious hepatitis whereas the recorded deaths are principally due to serum hepatitis which is well known to be a more severe disease

Human and Social Factors There appears to be little difference by sex or race in the incidence of infectious hepatitis in any age group though it may be mentioned that wherever the German Army went in World War II there the incidence of infectious hepatitis increased considerably i e Norway Russia the Balkans North Africa Italy and of course in Germany itself [1]

With regard to age it would appear that the highest rates for morbidity are in the age groups from 5 to 9 and 10 to 14 regardless of geographical location (Figure 4) [57] but the mortality tends to rise with increasing age (Figure 5) This is in line with the effect of other virus infections such as mumps measles and poliomyelitis [41]

There are several inexplicable slight statistical variations in sex dif

REPORTED MONTHLY INCIDENCE OF VIRAL HEPATITIS
PER 100000 POPULATION ADJUSTED TO AN ANNUAL BASE
UNITED STATES AND SCANDINAVIA
1952 — 1954

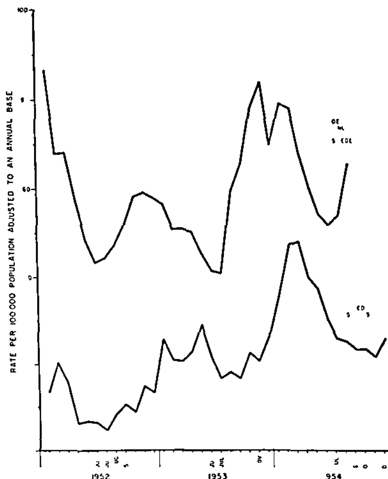


Figure 3 (F cm Sh rman and Eichenwald *Ann Int Med*)

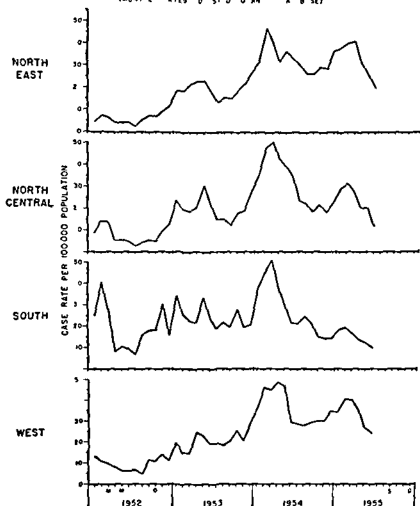


FIGURE 2 (From Sherman and Eichenwald *Ann Int Med*)



FIGURE 5 (From Sherman and Eichen ld *Ann Int Med*)

VIRAL HEPATITIS, AGE-SPECIFIC MORBIDITY RATES SELECTED STATES, 1953

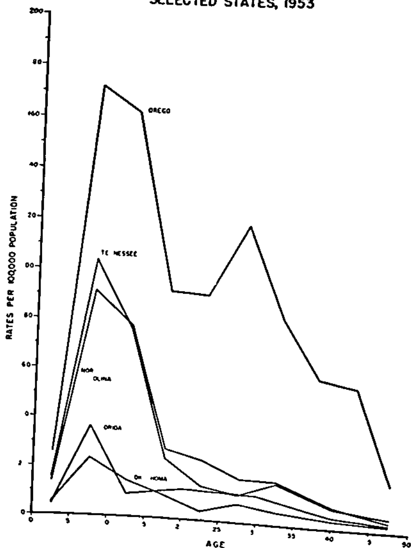


FIGURE 4 (From Sherman and Eichenwald *Ann Int Med*)

use of single personally cleaned mess kits by ordinary troops is much more sanitary than the communal mess arrangements of HQ and officers[1]

Biological Factors None are known

Yellow Fever

Pathological Factors

Causative Agent As far back as 1902 Reed and Carroll[50] had suggested that yellow fever may be caused by an ultramicroscopic organism but unfortunately Noguchi[4] in 1919 claimed that the causative agent was a Leptospira It was not until 1927 that Stokes *et al*[61, 6] demonstrated conclusively the viral nature of the disease when they isolated the now famous Asibi strain

Stokes and his co workers[6] found that the rhesus monkey was susceptible to the virus and later work confirmed its filtrability[5] the demonstration of antibodies in man and monkey and proof that the virus in Africa was the same immunologically as that found in South America[70, 5] In 1930 Theiler[68] found that the white mouse was a susceptible animal and on intracerebral inoculation an encephalomyelitis was produced which was transmissible to other mice but which became attenuated to lose its pathogenicity to rhesus monkeys These findings laid the foundation for a diagnostic test and the production of a vaccine

The virus in its natural state has an affinity for all three embryonal layers that is it is pantropic However modified strains have been found to have selective affinities for various specific embryonal layers and these can be increased by animal passage Thus some strains are viscerotropic (or hepatotropic) in the rhesus monkey whereas others are neurotropic in the mouse

Vectors Intermediate Hosts and Reservoirs It was believed that the epidemiology of yellow fever was simple and consisted of transmission of the virus from man to man by the house mosquito *Aedes aegypti* This was the form of yellow fever now known as epidemic or urban yellow fever affecting towns The host was man and the vector the mosquito and the disease was kept active by the influx of a nonimmune populace and a high population density of vector

However in 193 Soper *et al*[58] found yellow fever in a rural area in Brazil in the absence of *A aegypti* and it was soon realized that another epidemiological type of yellow fever existed in the tropical rain forest of the Amazon basin because of this feature Soper[9] named it jungle yellow fever It is also called sylvan or rural yellow fever This epidemiological type of yellow fever is a disease of the forest The

ferences in mortality The female during the reproductive period and particularly during the third trimester of pregnancy appears to be the more susceptible and the mortality rates of males over 50 years of age are in excess of those of females

The mortality from infectious hepatitis appears to be influenced by the state of nutrition This of course allows for a study of the geographic distribution of the disease generally speaking the areas of malnutrition tend to be the tropical underdeveloped countries of poor economy

Thus Findlay [0] found in West Africa that the mortality rate in West African troops was from 9 to 4 times that in United Kingdom troops despite the greater incidence of disease in the latter Further he found by comparing the mortality rates of the disease on two sides of a river in Nigeria that the higher rates were found in the community showing evidence of greater malnutrition

Fernando *et al* [18] and Fernando and Thanabalasunderam [17] in Ceylon from 1945 to 1950 studied 135 cases of hepatitis and 7 of cirrhosis (5 with a clear history of hepatitis) The mortality among the hepatitis cases was 18 5 per cent—i e almost 100 times that in civilian epidemics in the United Kingdom and United States

The dietary history was available in 97 cases and their work indicates that a poor diet influences the prognosis adversely as well as increasing the incidence of complications in patients with anemia and hypoproteinemia epidemic hepatitis had a poor prognosis

Straub and Schaberg [64] demonstrated histological changes indistinguishable from virus hepatitis in no less than 17 out of 60 livers obtained from Indonesians dying from hunger cachexia

Joe and Tjokronegoro [36] accepted viral hepatitis superimposed upon malnutrition as a cause for frequently occurring hepatic fibrosis in children in Java

One invariable feature of epidemics of infectious hepatitis is that the disease is prevalent in armies during wartime (Kriegsikterus) e g in Germany in World War I [4] and in World War II in the Middle East [60] Italy [41] Germany [48] and later Korea [7] In general diet was not inadequate and appears to have little relation to the incidence of the disease and it has been postulated that the prevalence of the disease is due to the herding together of large bodies of troops in which the infective nature (personal contact) of the disease can become manifest One interesting feature among the British was that headquarters troops showed a higher incidence than troops of the line and also that officers were more often affected than other ranks [60, 41] Although it is tempting to believe that the virus of infectious hepatitis and genius is found more frequently in officers and HQ troops for the same reasons the real explanation is probably that the

use of single personally cleaned mess kits by ordinary troops is much more sanitary than the communal mess arrangements of HQ and officers'

Biological Factors None are known

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vector (and possibly reservoir) is the blue mosquito (*Haemagogus*) which makes its habitat at treetop level

The reservoir is not exactly known but monkeys (e g, howler and spider monkeys) and possibly other animals and birds harbor the virus

It is known that monkeys are fatally affected in epizootics of yellow fever which pass as waves through a forest preceding the epidemic form of the disease in humans From these naturally affected monkeys the virus can be recovered as was shown in Trinidad recently by Anderson and Downs

Man The incubation period is 3 to 6 days and a clinical attack of yellow fever usually falls into three distinct phases the period of infection lasting about 2 to 3 days in which the virus is found in the blood the period of remission lasting from a few hours to 2 days and the period of (a) convalescence or (b) intoxication in which free virus is not found in the blood but an increasing amount of antibody can be demonstrated

In the phase of intoxication which may last from a few days to 2 weeks the classical symptoms of yellow fever show themselves in jaundice black vomit albuminuria indicating a profound toxemia Lins[39] has aptly described this the intoxication is everything the infection nothing or almost nothing The mortality is 5 to 10 per cent of all cases and no clinical relapse of yellow fever has been described

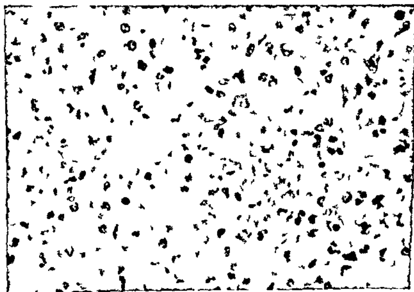


Figure 6 Liver from a case of acute yellow fever showing the central zone of a lobule with necrosis Councilman bodies (See text) (Hematoxylin and eosin × 135)

The virus in the liver produces a hepatitis involving 5 to 95 per cent of the parenchymal cells which show changes beginning with cloudy swelling and progressing to fatty metamorphosis and necrosis This necrosis is histologically an acidophilic cytoplasmic coagulation (Councilman bodies Figure 6) or a simple necrobiosis which classically is midzonal in situation, often with a centrolobular ring of intact cells[11] Inclusion bodies (Torres bodies) have been demonstrated in the nuclei of some human cases and nearly always in experimental rhesus monkeys infected with the disease[71]

In keeping with the behavior of many other viruses the yellow fever virus attacks primarily the parenchymatous cells in which parasitization and degeneration are the first events it would seem that the secondary cellular mesenchymal reaction is conspicuous by its mildness in contradistinction to the severe reaction often found in infective and serum hepatitis The hepatitis of yellow fever is perhaps an example of a true parenchymatous inflammation described by Virchow[5] As far as is known there is complete regeneration of the liver cell cords on recovery with no aftermath of fibrosis[37 7]

Geographical Factors

Figure 7 shows the world distribution of epidemics of yellow fever since 1933 and the areas in which the populace have been shown to contain neutralizing antibodies in their blood these latter are presumptive areas of endemic yellow fever[3 66 46]

The map also shows the world distribution of possible vectors In these days of air travel it takes but little imagination to conceive of the spread

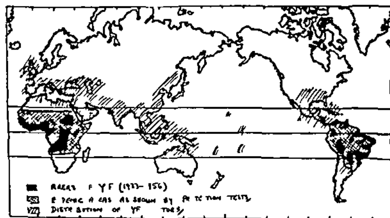


Figure 7 World map of yello fe er (Compiled from information supplied by WHO and Pan Am San Bur)

of the disease from the reservoir areas to unaffected areas of nonimmune populaces and suitable vectors if precautions are not taken

Physical Factors Yellow fever in its urban form involving the man mosquito cycle depends upon the year round survival of the aegypti mosquitoes Such conditions are found only in the suitable climatic conditions of tropical and subtropical countries as shown in Figure 7 In Europe and North America the winter temperatures are too cold for the survival of the mosquito and epidemics have only resulted when the virus has been introduced from a reservoir within the tropics However in the Mediterranean area the virus has been transmitted from one summer to the next as the result of mild winters[63]

In sylvan yellow fever the climatic factors are more complicated In general the vector is tropical and also is arboreal during the dry seasons the *Haemagogus* may be found only at treetop level descending in the rainy season for the next breeding It is of significance that the human cases of jungle fever tend to occur during the mid portion of the rainy season

With regard to the other part of the sylvan yellow fever cycle — the animal host — climatic conditions favoring tropical forests are necessary for the monkey populace Sylvan yellow fever advances epizootically and this may in part be explained by wind drift of infected mosquitoes into areas of nonimmune arboreal animals[1] Whatever the explanation of the spread it is probably a geographical factor affecting the breeding habits of both mosquito and monkey Thus oceans continental divides and deforested areas act as barriers preventing the spread of the disease unless they are crossed by human carriers

Human or Social Factors The social or human factors affecting yellow fever are well illustrated in the spread of yellow fever from the tropical areas of South America and Africa to Europe and North America during the last three centuries (Figure 8)[1] This was the era of urbanization of the disease The major sources of infection were the holds of ships in which hordes of mosquitoes were trapped and subsequently bred these mosquitoes were infected with virus at the point of origin or by the passengers or crew in transit At arrival at the point of destination the infected mosquitoes swarmed out avid for a blood meal and caused not inconsiderable epidemics in Spain 6 000 deaths in 1800 in Italy 6,5 deaths in 1805 in the Mississippi Valley (Memphis) 5000 deaths in 1878 Luckily these epidemics were localized to the ports and seaboards and were generally terminated by the effect of the climate on the vector

In sylvan yellow fever the human cases are rural dwellers engaged in farming at the edge of the forest areas or within the forest clearings The

FIGURE 8 Spread of yellow fever from reservoir areas (Compiled from information supplied by Elton)

Haemagogus seeks a blood meal at about midday which is the time the farmer is active in the fields or felling trees and this is the resting or napping period of the monkey These factors favor the transmission of the virus [12]

Biological Factors These have been dealt with in the previous sections on geographical factors but a further illustration can be made by a consideration of the current wave of yellow fever in Central America

In 1948 like a bolt from the blue there was a sudden outbreak of sylvan yellow fever on the Pacific side of the Great Divide This outbreak subsequently has spread westward to reach its most northern point in Guatemala in 1956 (Figure 9)

From the onset the wave spread from the Pacific to the Atlantic side of the Great Divide via the Panama Canal gap and has continued northward along the Atlantic rain forests until the present time with two exceptions The first was in 1951 when the disease unexplicably arose on the Pacific side of the Great Divide in Costa Rica it is believed that the mountain barrier was crossed by human carriers who had been infected by the main wave in the north The second was the passage of the wave through the natural gap in the Great Divide barrier occasioned by the Lake Nicaragua in 1952

The wave has passed northward at a velocity of about 11 to 13 miles per month and below the 000 foot contour generally around the 1000-foot mark The front of the wave appears to be epizootic with the human

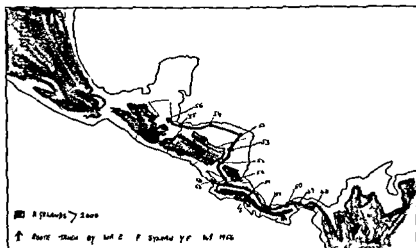


FIGURE 9 Current wave of yellow fever in Central America 1948–1956 (Compiled from information supplied by Elton and Pan Am San Bur)

epidemic phase lagging behind by several months however at times these phases may coincide The epizootic waves have involved the arboreal primates with a high mortality among howler spider and marmoset monkeys The epidemics in the human population have frequently occurred at the time of the heavy rains appear to be self limiting and rarely last more than two months This is thought to be related to the life span of the vector *Haemagogus spegazzinii*, which is about 65 days The wave propagation appears to be due to wind drift of the infected mosquitoes into areas populated by nonimmune monkeys it has reached Northern Honduras (La Ceiba) and Guatemala (Lake Izabal) and forecasts have been made that the wave will extend further northward into Mexico [16 1 1 13 14 46]

VENOUS OCCLUSIVE DISEASE OF THE LIVER

Pathological Factors

This is a disease of the liver in which the primary lesion appears to be the occlusion of the smaller hepatic veins The occlusion resembles to some extent endophlebitis hepatica obliterans [10 07]

Causative Agent This is not known in the human although there is some suggestive evidence that Senecio poisoning in cattle [43] is a similar disease It has for a long time been suggested that the drinking of bush teas which are widely taken in Jamaica for their medicinal value may also play an important role because of their hepatotoxic properties [4]

The disease may also be associated with a low protein diet [51, 8] which would reduce the reserves of the liver to deal with any hepatotoxic factor or it may be related to an infection which often precedes an acute attack [9] The infection is generally of the respiratory tract and often bush teas are taken as a remedy for such complaints [67] It is thus uncertain what part the infection or the bush tea has to play in the production of the disease

Although extensive studies have been done on the toxicity of bush teas by Gyorgy and our group to date the disease has not been reproduced in an experimental animal The hepatotoxic properties of some bush teas have however been established

Vectors Intermediate Hosts, Reservoirs There are none

Man The disease is predominantly seen in children aged 2 to 5 years although adults are also affected Three clinical stages have been defined [30, 9, 34] an acute stage characterized by acute ascites and hepatomegaly (Figure 10) the subacute stage and the chronic stage in which there is cirrhosis of the liver

Histologically [8] the essential finding in the acute stage is a blockage of the medium sized and smaller hepatic veins by a subendothelial swelling of the intimal tissues (Figures 11 and 12) This swelling is apparently due

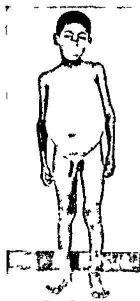


FIGURE 10 Acute V O D with ascites and hepatomegaly 6-year-old male



FIGURE 11. Liver from a case of acute V.O.D., showing occlusion of centrolobular veins and back pressure distension of sinusoid. (Hematoxylin and eosin × 35.)

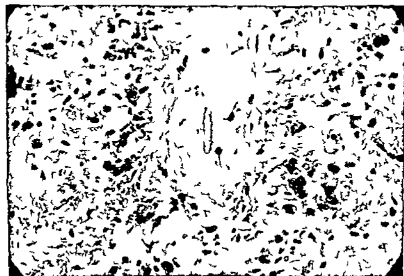


FIGURE 12. H.P. of Fig. 10 showing endothelial swelling of centrolobular vein. (Hematoxylin and eosin × 1[illegible]0.)

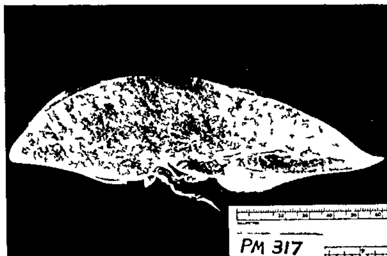


FIGURE 13 Liver from a case of acute V O D showing an appearance similar to the "nutmeg" pattern of cardiac venous congestion

to edema but later becomes collagenous This intimal occlusion produces a block centrolobularly with distension of the tributary sinusoids so that macroscopically the appearance resembles that of a nutmeg liver of cardiac congestion (Figure 13)

The subacute stage shows a persistence of centrolobular fibrosis (non portal) and the chronic stage is that of a fully developed nonportal cirrhosis

Geographical Factors The geographical distribution of venous occlusive disease of the liver in human and animal cases is given in Figure 14 and appears to be world wide

Human cases have been extensively studied not only in Jamaica[4, 30, 3, 8, 33, 34, 51, 7] but also in Barbados[6]

In India Rao (1935) has described an infantile cirrhosis originally said to be a form of biliary cirrhosis Rao demonstrated venous occlusion and after perusal of the literature the present writers believe that the condition may well be due to venous occlusive disease This however is not generally accepted by the Indian workers[1] Jelliffe and Bras[3] on the other hand have recently investigated some cases of Indian infantile cirrhosis the histopathology of which has been interpreted as similar to venous occlusive disease South African workers have suggested that Senecio (ragwort) poisoning may exhibit the picture of Chiari's syndrome in which thrombosis of the hepatic veins is one of the outstanding le

sions [6] and recently Higginson [8] has reported some cases of venous occlusive disease In Egypt Hashem has described a condition which we believe to be venous occlusive disease histologically although Hashem differed in his interpretation

In animals livers have been examined from cows in Jamaica exhibiting the classical lesions of venous occlusive disease although the exciting agent believed to be a weed has not yet been identified [6]

In England Markson [43] has investigated presumptive Senecio poisoning in cows and the lesions are those of venous occlusive disease [6, 7]

Enzootic liver cirrhosis demonstrated in horses and cattle in South Africa by Theiler [69] appears to be identical to venous occlusive disease while specimens sent to us by Dr Bull [9] from Australia from a horse suffering from Crotalaria poisoning revealed a similar histopathology

Human and Social Factors In all human cases no definite etiological agent has been identified other than the taking of bush teas in Jamaica or suggestive Senecio poisoning in South Africa However patients have belonged to a low income group and as a consequence there seems to be a relation with malnutrition and a low protein diet

In the animal cases (horses and cattle) the disease appears to be associated with the eating of poisonous weeds either of the Senecio or Crotalaria groups (pyrollizidine alkaloids)

There appears to be no significant sex or racial incidence although the disease may be conditioned by the prevalence of a low economic status in colored peoples

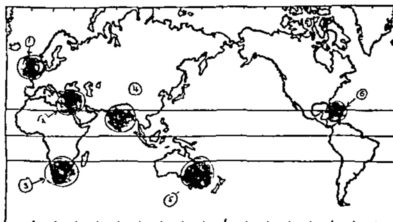
Biological Factors Little research has been done of any significance into the susceptibility of different blood groups Many human patients are highly parasitised e g ascariasis and hookworm and although these are probably not related to the primary cause of the disease they may play a secondary role

SUMMARY

Geographic pathology is the interaction of pathological and geographical factors these are discussed as they affect viral hepatitis yellow fever and venous occlusive disease

In viral hepatitis (infectious and serum varieties) there appears to be a seasonal incidence in colder climates but not in the tropics Certain areas such as Germany and the Mediterranean littoral appear to be seeded by the disease malnutrition which is a geographic factor has an effect on prognosis

In yellow fever geographical and pathological factors play a great part in the spread of the virus in both the urban — mosquito man cycle



1 U K (C ttl) 3 South Afr a (H ma horse attl) 5 Aust l a (H)
2 Egypt (H m s) 4 I d (H m) 6 We t I d s (H ma cattl)

FIGURE 14 World distribution of V O D (Compiled from information supplied by WHO and Pan Am San Bur)

— and the jungle — mosquito monkey (man) cycle — types of disease The current wave of yellow fever in Central America is discussed illustrating these points

In venous occlusive disease to date only the pathological factors have been extensively studied and although the disease has a world wide distribution the importance of the geographic aspect is not yet apparent

ACKNOWLEDGMENTS

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5

*Pathologic Anatomy of Early Stages of Viral Hepatitis**

HANS F SMETANA MD
(Washington D C) **

Histopathologic examination of the liver is the only means of diagnosing viral hepatitis with reasonable accuracy While clinical observations and the results of pertinent laboratory tests [10 4 6–8] often yield strong evidence for or against this diagnosis they do not furnish reliable proof Even during epidemics of viral hepatitis when it might be expected that the clinical diagnosis of individual cases could be made with comparative assurance biopsy studies have proved the existence of some other infection *in many instances* [69 71] *In sporadic cases clinical diagnosis presents even* greater difficulties and the value of biopsy is emphasized for such cases may be the forerunners of an impending outbreak of epidemic proportions

The histopathologic changes occurring in infectious hepatitis and homologous serum hepatitis are identical even though distinct albeit related viruses [23 4 0 3] have been established as the etiologic agents of the two diseases It is in the several stages and phases of viral hepatitis of both types that great differences are obvious in the alterations of liver tissue Recognition of the disease is comparatively easy in its early stages of florid change but is increasingly difficult in the subacute and chronic stages unless biopsies have been performed early in the course of the disease

Because evaluation of the changes in liver tissue during the early stages of viral hepatitis is so essential to diagnosis the purpose of this paper is to present the detailed criteria that have been established for the diagnosis of this condition particularly in needle biopsy material which has proved to be most satisfactory for histopathologic study [34 6 69 71]

* With particular reference to the epidemic in Delhi India 1955–1956

** Present address The Vallabhbhai Patel Chest Institute Delhi University Delhi India under the auspices of the U S International Co-operation Administration Technical Co-operation Mission Health Division New Delhi India

Histopathologic Changes During the Acute Stage of Nonfatal Viral Hepatitis

In biopsy material obtained soon after the appearance of clinical symptoms of hepatitis the lobules of the liver exhibit a variety of changes which however do not significantly alter the essential lobular architecture The normally quiescent liver cells exhibit marked unrest and great variation in size and shape the radial columns often deviate from their usual straight course and are irregular in width (Figures 1 and) Infiltrates consisting mainly of mononuclear cells occupy portions of the sinusoids and of the columns Many of the Kupffer cells contain granular golden brown pigment (Figure 3) which in all probability is lipofuscin [9, 0, 33, 40, 64–66, 69, 78] The portal triads are unusually prominent because their stroma is diffusely infiltrated by mononuclear cells and eosinophils (Figure 4) Despite the presence of severe clinical jaundice and high titers of serum bilirubin retention of bile within the bile canaliculi or small bile ducts is not a feature in the early stages of hepatitis of this type [33]

Alteration of individual liver cells consists of swelling or shrinkage (Figures 3 and 5) Swelling is associated with vacuolation of cytoplasm and partial or apparent reduction of the cytoplasmic granular material that usually can still be recognized about the nucleus but sometimes remains entirely unstained The nucleus may be without significant changes except perhaps slight swelling and the presence of eosinophilic nucleoli The cell membrane is clearly defined in instances of extreme ballooning of liver cells it encloses a space showing but thin threads of a cytoplasmic web about a centrally located nucleus [5, 33, 43, 69] Occasional granules of lipofuscin are seen in some of the cells thus altered

Shrinkage of liver cells is accompanied by eosinophilic hyalinization of the cytoplasmic contents with pyknosis or karyorrhexis of the nucleus (Figures and 3) The hyalinized material or so-called acidophilic body [60, 63] becomes detached from the cell membrane and is discharged into the sinusoids Formation of multinucleated giant cells from liver cells also occurs their cytoplasm may contain lipofuscin and occasional particles of bile

In addition to degenerative changes of liver cells regeneration is suggested by mitoses of nuclei or multinucleated cell elements Actual necrosis of liver cells is rarely seen but must be assumed even though no shadows of dead or dying liver cells remain and no ghost cells mark the local effect of the virus infection

In most instances these alterations affect the liver lobules diffusely [5, 33, 69] although the centrolobular areas are perhaps more severely damaged than others (Figure 1) Needle biopsies from any portion of the organ reveal the same significant changes [9, 77]



P p rt l l E ff t

FIGURE 1 Acute nonfatal viral hepatitis Liver needle biopsy 3 days after onset of illness Moderate irregularity of liver cells, with degenerative forms focal mononuclear cell infiltrates within the lobules and in portal areas and some distortion of the radial cell columns Diffuse involvement of lobules (× 115) (AFIP Accession 3 704)

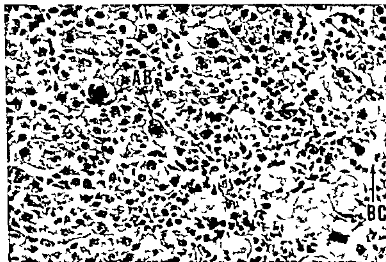


FIGURE 2 Acute nonfatal viral hepatitis Liver needle biopsy 3 days after onset of jaundice Striking alteration of liver cell with formation of balloon cells (BC) and early acidophilic bodies (AB) Architecture of lobule distorted by irregularity of liver cells forming the columns Intralobular mononuclear cell infiltrates and focal congestion of sinusoid Lipofuscin in some Kupffer cells (× 325) (AFIP Accession 325047)

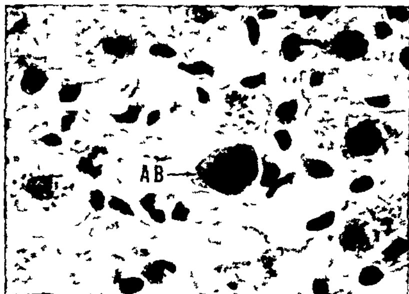


FIGURE 3 Acute nonfatal viral hepatitis Liver biopsy 3 days after onset of illness Degenerative changes of liver cells with formation of balloon cells and acidophilic body" (AB) Lipofuscin in Kupffer cells (K) A few mononuclear cells are seen in sinusoids (X 900) (AFIP Accession 32704)

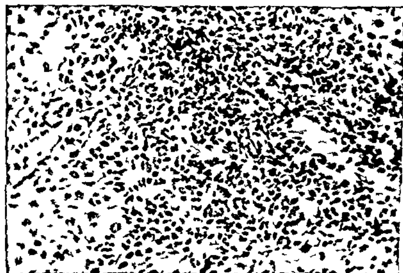


FIGURE 4 Acute nonfatal viral hepatitis Liver needle biopsy 3 days after onset of illness Extensive infiltrates composed of mononuclear cells, lymphocytes and eosinophils in stroma of portal canals (X 300) (AFIP Accession 327042)

Stains for reticulum show that the individual fibers are intact but that collapse of the stroma and condensation of argentophilic fibers have oc curred in areas of the parenchyma where damage is severe (Figure 6) The biliary passages do not exhibit any characteristic alterations Neither the portal vessels nor the efferent veins seem to be damaged in nonfatal cases in some fatal cases however necrosis of the walls of efferent veins sometimes associated with centrolobular infiltrates has been described

Fat in liver cells is so rarely seen in this disease that its obvious presence speaks against the diagnosis of viral hepatitis[17, 33, 66, 69] unless the patient has been vigorously treated with antibiotics or hormones

No single change is diagnostic of viral hepatitis the individual features vary greatly in different cases and in different stages and phases of the same case Not all of the changes enumerated need be present at all times Some are but scantily represented others may dominate the picture and thus tend to obscure other details It cannot be too strongly emphasized that the important diagnostic feature of acute viral hepatitis is this very kaleidoscopic and variegated nature of the change that involves every lobule throughout the entire liver altering completely its regular quiescent appearance (Figure 7)

This applies equally to the histopathologic appearance of nonfatal viral hepatitis as seen in liver needle biopsies in Europe[5, 9, 15, 67, 74, 77] including the Scandinavian countries[9, 31, 38, 60] the Mediterranean[8] North Africa[7] the Middle East[2, 46] the Americas[40, 43, 63] Japan[13] and Korea[13] According to Wahi[75] the lesions observed in an epidemic in Agra India conform in general to those seen in other parts of the world

Interpretation of the Changes Seen in the Acute Stage of Nonfatal Viral Hepatitis

The basic alteration of the liver resulting from infection with the virus of hepatitis is one of damage to individual cells leading either to degenera tive changes or to necrosis Although necrosis of liver cells is rarely seen indirect and circumstantial evidence that it occurs is provided by collapse and condensation of the reticulum fibers in areas of massive destruction of the parenchyma and focal distortion of the course of these fibers adjacent to altered cell columns Focal infiltrates of mononuclear cells within the lobules probably represent local reactions about necrotic liver cells even though the affected cells are no longer visible

Extensive centrolobular necrosis a prominent feature in acute yellow atrophy is not observed even in severe hepatitis The cellular infiltrates sometimes seen centrally in fatal cases of hepatitis have been interpreted as an inflammatory reaction about the efferent vein These cells most of which are mononuclear and some of which contain lipofuscin may simply represent wandering cells that are carrying phagocytized debris from the

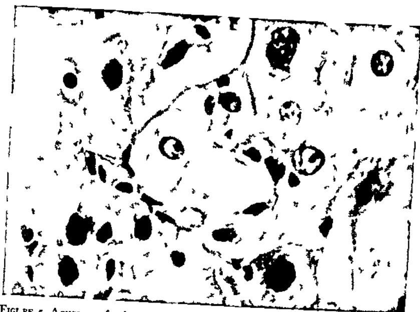


Figure 5 Acute nonfatal viral hepatitis Liver needle biopsy 5 days after onset of illness Great variation in size shape and staining characteristics of liver cells Some are swollen others have formed balloon cells Occasional binucleated elements are present (× 960) (AFIP Accession 316131)

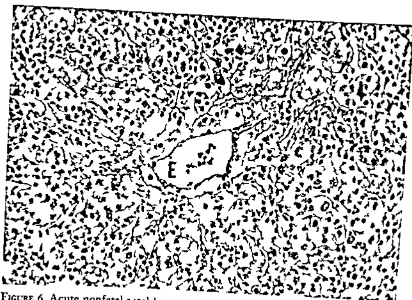


Figure 6 Acute nonfatal viral hep titis L er needle biopsy 23 days after onset of jaundice Center of lobule showing efferent vein (E) and radiating cell columns The reticular framework is preserved but collapse and cond n ation of argentophilic fibers in many areas indic te loss of liver cells and cell columns (Wilder reticulum stain × o) (AFIP Access on 3 504)

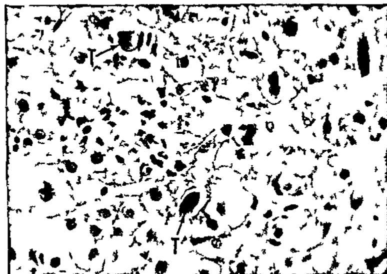


FIGURE 7 Acute n nfatal viral hepatitis Liver needle biop y 21 days after onset of illne s Focal necroses in l bule Bile thrombi (T) in bile capillaries (× 525) (AFIP Accession 319263)



FIGURE 8 Pers stent n nfatal ral hepatit s th po tnecrotic scarring Liver needle b op y 5 1 ntl after n et f illness Enlargement and scarring of p rt l area (P) w th exten n t ne ght ring p rtal fields. Mononuclear cell infiltrat in str ma of tri d and prol ferat ng ile ducts The scars d ide the l bul nt rregular p t o some of which contain an efferent vein (E) (× 5) AFIP A n 563007)

necrotic lobules to the nearest vessel. The presence of lipofuscin in Kupffer cells which ordinarily are devoid of this pigment is construed as further evidence of the destruction of liver cells that contained lipofuscin with subsequent phagocytosis of pigment granules. Lipofuscin may also be seen in the acidophilic bodies.

The presence of lipofuscin in altered but non necrotic liver cells and in acidophilic bodies and its absence in newly regenerating cells suggest that this pigment within a liver cell indicates degenerative change. Evidence that these altered cells have disintegrated is the lipofuscin in phagocytes.

In general alterations of liver cells in this stage of hepatitis are regarded as reversible for there are cells undergoing mitosis and the familiar multinucleated regenerating cells as well as swollen or even balloon cells that still are viable. Acidophilic bodies which are the products of more advanced degeneration are not regarded as viable cells.

The intrasinusoidal infiltrates of mononuclear cells appear at times as if they might have sprung from local reticulum cells that possess potential hematopoietic function[5] while similar accumulations of cells in the portal areas represent true infiltrates. The eosinophils may reflect either a systemic eosinophilia or a local leukotaxic effect. The portal infiltrates are not interpreted as an expression of a focal inflammatory reaction or triaditis.

The absence of retained bile in the canaliculi in the presence of severe *clinical jaundice and significant cellular alteration of the hepatic paren*chyma is believed to be the result of extravasation of bile into tissue spaces and sinusoids from the canaliculi disrupted by the necrosis of parenchymal cells. For this reason the absence of bile thrombi cannot be taken as evidence of the existence of anicteric hepatitis[1, 5, 8, 33, 49, 60]

The remarkably good preservation of the reticulum fibers despite some distortion even in severe or fatal cases is very likely responsible for the perfect realignment of the liver cells as they rebuild the depleted cell columns.

The Histopathologic Changes in Subsiding Viral Hepatitis

Comparison of liver biopsy material from convalescent patients with that obtained during the florid stage of the disease shows a gradual diminution of necrotizing and degenerative alterations of the hepatic parenchyma with regenerative changes predominating. There are still irregularities in the size and shape of liver cells as well as in the width and course of many of the cell columns. It is during this phase of subsiding hepatitis that histologic evidence of bile retention may appear. The mononuclear cell infiltrates particularly within the sinusoids diminish in extent and gradually disappear those in the stroma of the portal spaces usually remain for

a longer time before they too vanish. For some time afterward the portal canals appear larger than normal and seem scarred. The lipofuscin in the Kupffer cells remains visible for several weeks or months and in the later phases may be associated with stainable iron.[66, 69] The reticulum fibers tend to resume their normal relations with liver cells and sinusoids and areas of collapse and condensation are less frequently encountered. Finally the liver tissue regains its normal appearance[41, 69] and laboratory tests indicate restitution of the normal function of the organ.

Interpretation of the Histopathologic Changes in Subsiding Viral Hepatitis

The evidence of continuing restoration of liver tissue indicates that the infection is being overcome and normal hepatic functions are being resumed. As necrosis of liver cells ceases, infiltration of mononuclear cells subsides. The walls of the disrupted bile canaliculi are restored as the cords of liver cells are re-formed; thus the bile cannot spill out as it did during the stages of liver cell destruction. The plugging of canaliculi that is now demonstrable is regarded as a by-product of regeneration of parenchymal cells. The newly formed cells, often larger than their mature fellows, may cause temporary obstruction and stasis of bile in the disrupted canaliculi. The logical discrepancy in trying to establish a causal relationship between bile thrombi and clinical jaundice is readily revealed at this stage, for even as the bile thrombi are increasing in number the serum bilirubin is dropping and the jaundice is subsiding.

The fate of the lipofuscin phagocytized by the Kupffer cells is not known, but the pigment disappears from these cells after several weeks or months. During the period of restoration of liver cells, iron pigment is usually demonstrable in Kupffer cells, an indication, perhaps, that the regenerating cells are not yet equal to their many functions, including their role in iron metabolism.

Full recovery from viral hepatitis is generally associated with *restitutio ad integrum* of the parenchyma of the liver.

The Histopathologic Changes Associated with Postnecrotic States of Viral Hepatitis

Much has been written on postnecrotic states of viral hepatitis.[4, 7, 15, 39, 44, 4, 6, 65, 69, 8]

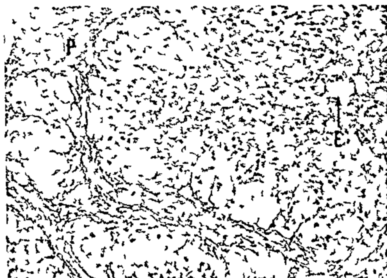
Simultaneous destruction of large portions of the liver parenchyma by the virus may create a situation in which the effective replacement of liver cells is retarded and incomplete. This is particularly true at the center of areas of extensive necrosis that often extend into neighboring lobules. In such areas there are no regenerating liver cells to separate the collapsed reticulum fibers and they remain as scarlike condensed strands of argento

philic fibers[9, 1, 69] traversing the lobules in haphazard fashion Different lobules are affected in different ways and despite the destruction of the surrounding cells many of the efferent veins are preserved and are seen in their usual position (Figures 8 and 9) In addition to these changes the liver lobules exhibit many or all of the alterations ordinarily seen in viral hepatitis

Almost complete destruction of the liver parenchyma in a severe near fatal attack of viral hepatitis may be followed by undisturbed regeneration and reorganization of liver tissue A possible consequence of such a course is the formation of coarse nodular cirrhosis or postnecrotic cirrhosis[68] (Figure 10) Although sufficient liver tissue remains to support life the altered architecture of the lobules gives rise to circulatory disturbances which eventually result in portal hypertension and its sequelae (Figure 11) This condition is a very rare extreme consequence of viral hepatitis and can be distinguished from cirrhosis of other types by its specific characteristics[68, 69]

Interpretation of the Changes Seen in the Postnecrotic States of Viral Hepatitis

The histapathologic picture seen in biopsy specimens from cases of postnecrotic collapse is but an exaggeration of that usually encountered in ordinary viral hepatitis except for the more extensive simultaneous necrosis of large groups of liver cells Restoration of the functional organization of the lobules is incomplete and accompanied by apparent permanent collapse and condensation of reticulum fibers to simulate scars The distorted pattern in the regenerating liver may be mistaken for that of portal cirrhosis particularly in needle biopsies where only a limited portion of the parenchyma can be surveyed However further biopsy studies will show that the scarring is neither general nor does it alter the structure of all the lobules Cirrhosis connotes a general diffuse and uniform alteration of the structure of the liver with reorganization of regenerated liver cells into pseudolobules The local fibrosis and scarring or condensation of reticulum in hepatitis cannot be reconciled with this definition[69, 1] neither are these changes succeeded by the physiologic consequences of long standing cirrhosis portal hypertension development of collateral circulation and eventual hepatic insufficiency In some examples of extensive postnecrotic scarring (coarse nodular cirrhosis) the terminal physiologic effects on the patient may be indistinguishable from those of portal cirrhosis but the pathogenesis and histogenetic development of the two conditions are entirely different In a study of cases in which there was a history of viral hepatitis cirrhosis could not be attributed solely to the effects of the hepatitis in a single case[7] It is possible that postnecrotic scarring has in some instances been identified



P pot l tr d E ff e t e

FIGURE 9 Persistent nonfatal viral hepatitis with postnecrotic scarring Liver needle biopsy 5 months after onset of illness Condensation of reticulum fibers between preserved portions of liver tissue and about groups of regenerated liver cells (Wilder reticulum stain × 100) (AFIP Accession 563007)

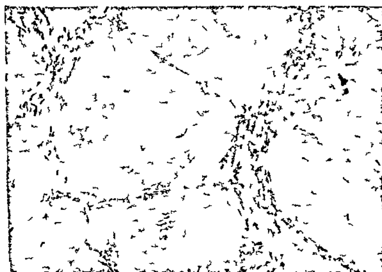


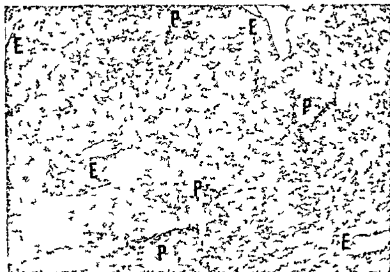
FIGURE 10 Coarse nodular cirrhosis of liver The individual nodules are composed of regenerated and reorganized but poorly defined lobules The nodules are separated by septa formed mainly of collapsed and condensed reticulum fibers and the stroma of surviving portal canals with its bile ducts and portal vessels (× 14) (AFIP Accession 160804)

philic fibers[9, 13, 62] traversing the lobules in haphazard fashion. Different lobules are affected in different ways, and despite the destruction of the surrounding cells many of the efferent veins are preserved and are seen in their usual position (Figures 8 and 9). In addition to these changes the liver lobules exhibit many or all of the alterations ordinarily seen in viral hepatitis.

Almost complete destruction of the liver parenchyma in a severe, near fatal attack of viral hepatitis may be followed by undisturbed regeneration and reorganization of liver tissue. A possible consequence of such a course is the formation of coarse nodular cirrhosis or postnecrotic cirrhosis[65] (Figure 10). Although sufficient liver tissue remains to support life, the altered architecture of the lobules gives rise to circulatory disturbances which eventually result in portal hypertension and its sequelae (Figure 11). This condition is a very rare, extreme consequence of viral hepatitis and can be distinguished from cirrhosis of other types by its specific characteristics.[68, 69]

Interpretation of the Changes Seen in the Postnecrotic States of Viral Hepatitis

The histopathologic picture seen in biopsy specimens from cases of postnecrotic collapse is but an exaggeration of that usually encountered in ordinary viral hepatitis, except for the more extensive simultaneous necrosis of large groups of liver cells. Restoration of the functional organization of the lobules is incomplete and accompanied by apparent permanent collapse and condensation of reticulum fibers to simulate scars. The distorted pattern in the regenerating liver may be mistaken for that of portal cirrhosis, particularly in needle biopsies, where only a limited portion of the parenchyma can be surveyed. However, further biopsy studies will show that the scarring is neither general nor does it alter the structure of all the lobules. Cirrhosis connotes a general diffuse and uniform alteration of the structure of the liver with reorganization of regenerated liver cells into pseudolobules. The local fibrosis and scarring or condensation of reticulum in hepatitis cannot be reconciled with this definition,[71] neither are these changes succeeded by the physiologic consequences of long standing cirrhosis: portal hypertension, development of collateral circulation and eventual hepatic insufficiency. In some examples of extensive postnecrotic scarring (coarse nodular cirrhosis) the terminal physiologic effects on the patient may be indistinguishable from those of portal cirrhosis, but the pathogenesis and histogenetic development of the two conditions are entirely different.[1] In a study of cases in which there was a history of viral hepatitis, cirrhosis could not be attributed solely to the effects of the hepatitis in a single case.[17] It is possible that postnecrotic scarring has in some instances been identified



E ff r t v ns

FIGURE 11 Coarse nodular cirrhosis of liver Needle biopsy specimen of nodule showing several small portal triads (P) containing practically no stroma and many collecting sinusoids irregularly scattered about The radial arrangement of the lobules is being re formed (× 75) (AFIP Accession 693162)



FIGURE 12 Persistent nonfatal viral hepatitis Liver needle biopsy 38 days after onset of illness The liver cells are fairly uniform with but few multinucleated elements The portal infiltrate are scanty but occasional focal necroses are scattered through the lobules (× 45) (AFIP Accession 329833)

as cirrhosis of the liver because the term cirrhosis is so loosely used.

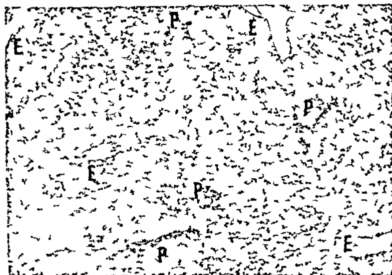
It is hoped that further well controlled studies will clarify the issue and that adoption of the terminology proposed by the Board of Classification and Nomenclature of Cirrhosis of the Liver will obviate future misunderstanding.[11]

The Histopathologic Changes in Subacute and Persistent Viral Hepatitis

A relatively small percentage of patients with hepatitis continue to suffer from indigestion, gastrointestinal discomfort and vague pains and sensations of fullness in the region of the liver long after the acute attack has passed.[3, 3, 4, 51] The results of liver function tests are somewhat abnormal.[45, 49] Needle biopsies of the liver performed at intervals after the acute episode show that the extent of the damage is diminishing and the original functional pattern of the lobules is being restored (Figure 12). Focal necrosis is still represented by the groups of mononuclear cells replacing liver cells which have disappeared from the columns (Figure 13). Occasional bile canaliculi contain bile thrombi but retention of bile is not general. The stroma of the portal areas remains moderately infiltrated by mononuclear cells and a few eosinophils. The most impressive feature, however, is the persistent inequality of individual liver cells. Many cells contain two, sometimes more, nuclei; differences in their sizes and shapes and in the staining properties of their cytoplasm impart a certain irregularity and appearance of unrest to the normally regular and quiescent radiating columns. An occasional "acidophilic body" may still be encountered. Rather striking are the "colonies" of Kupffer cells with the golden brown pigment of lipofuscin either granular or diffuse in their cytoplasm (Figure 14). These nests of pigmented cells are sometimes the predominant feature in parts of the lobules but have no apparent predilection for any particular portion. Phagocytes within the stroma of the portal canals may also contain this pigment. The reticulum fibers at various sites in the lobules are often condensed but the individual fibers remain intact and delicate. The stroma of the portal triad, partially depleted of mononuclear infiltrates, often exhibits a peculiar coarseness and the limiting membrane is not as clearly defined as in normal conditions (Figure 15).

Interpretation of the Changes Observed in Subacute and Persistent Viral Hepatitis

Persistence of the hepatitis in these cases indicates the possibility of tissue resistance with immediate fixation of the virus wherever it attacks the tissues anew. Yet definite if restricted activity of the virus is shown by the presence of focal necroses in the subacute and persistent disease



E ff t ns

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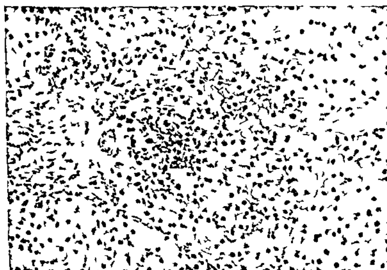


FIGURE 12 Persistent nonfatal r l hep t t I r ne dle bi psy 38 days after on et f lines The li r ll r t rl un f rm ith but few multi n cl t l elements The p t l i filtrat ar ts l ut occa i nal f cal necroses are scattered thro gh th l l l (4) (AFIP Accession 329833)

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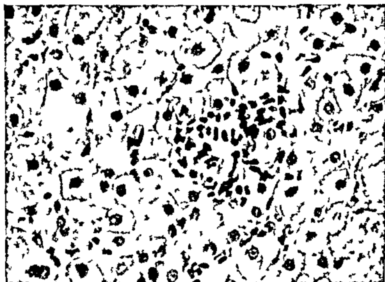


FIGURE 13. Persistent nonfatal viral hepatitis. Liver needle biopsy 24 days after onset of illness. Focal infiltrates of mononuclear cells about areas of necrosis of liver cells within the lobules. Slight irregularity of parenchymal elements (× 450.) (AFIP Access. n 320(43.)

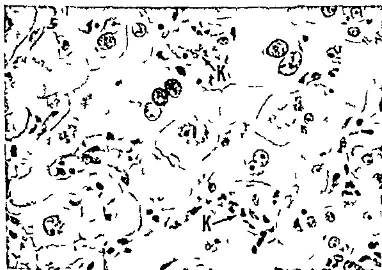


FIGURE 14. Persistent nonfatal [illegible] hepatitis [illegible] needle biopsy 30 days after onset of illness. [illegible] Kupffer cells (K) holding lipofuscin. Slight irregularity [illegible] cells [illegible] many multinucleated elements. (× 900.) (AFIP A[illegible] 60 686.)

The presence of occasional bile thrombi is probably related to regeneration of liver cells and repair of disrupted bile passages. The numerous Kupffer cells that contain lipofuscin suggest that injury to the hepatic parenchyma was actually more extensive than suggested by the number of visible focal necroses alone.

Viral hepatitis in which periods of clinical activity are followed by intervals of temporary quiescence has been known to persist for a long time, even for many years. Although the virus of hepatitis has not as yet been conclusively demonstrated in the blood or tissues of patients with the persistent disease,[51] the appearance of the liver in needle biopsies suggests no other cause for the histopathologic alterations, which are entirely compatible with those of viral hepatitis.

The rather unusual chronicity of this virus disease suggests an analogy with the persistent variety of oral herpes infection,[55] in which a state of unstable equilibrium between the tissues and the infectious agent is occasionally maintained through the years.

The concept that portal cirrhosis may be a consequence of persistent viral hepatitis[4, 6, 7, 1, 35, 5, 6, 5] has not been generally confirmed.[2, 41, 6, 7] In a study of 1000 cases of viral hepatitis at the Armed Forces Institute of Pathology from 1949 to 1955,[57, 59] histopathologic evidence of portal cirrhosis was never observed in repeated biopsies performed on patients with hepatitis that had persisted for many years.

Fatal Viral Hepatitis (Acute Yellow Atrophy, Subacute Red Atrophy, Catarrhal Jaundice, Epidemic Jaundice, Fulminant Hepatitis)

The extreme form of viral hepatitis was first accurately described by Rokitansky,[61] who gave it the name "acute yellow atrophy," and later by Rossle. This was also described by Lucke and Mallory as fulminant hepatitis.[4] The liver is found to be shrunken, flabby and a peculiar ocher yellow in color. This form of hepatitis is rapidly fatal; patients often die within a few days with signs of hepatic insufficiency. In general, length of survival is related to the amount of tissue spared by the virus and the speed of regeneration of liver cells.

The microscopic picture is characterized by extensive necrosis and the disappearance of liver cells, leaving only the stromal framework with its blood vessels and bile ducts (Figures 16, 17, and 18). The extent of the inflammatory reaction depends on the length of survival of the patient. It consists mainly of infiltrates of mononuclear cells, usually in and around the portal areas but occasionally about the efferent veins. In the latter location some of the cells may contain phagocytized granules of lipofuscin. The reticular stroma is well preserved; the individual fibers are intact. Superficially the lobular architecture appears unchanged except that the

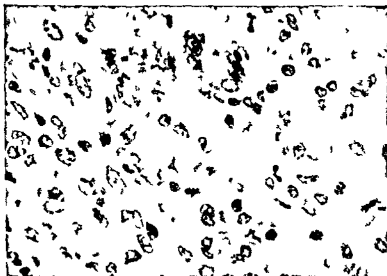
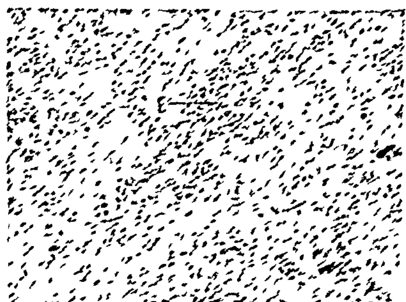


FIGURE 17 Acute fatal viral hepatitis Death 20 days after onset of illness Total necrosis of liver cell Surviving stromal elements phagocytic cells and bile ducts (× 450) (AFIP Accession 548232)



FIGURE 18 Acute fatal viral hepatitis D[illegible] [illegible] after onset of illness. Bile d[illegible] and [illegible] embedded in reticular framework (Wilder reticulum stain × 450) (AFIP A[illegible] 548232)





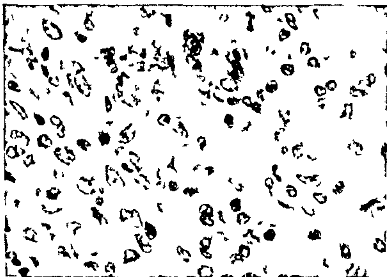


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FIGURE 18 Acute f t l r l hep tit Death 20 da after nset of illness. B le ducts and x a n l l r ll embedd l in ret cular framework (Wilder ret culum stain × 450) (AFIP Acce ion 548 32)

various stages of viral hepatitis Normal liver tissue was encountered in 3 instances infantile cirrhosis and fatty liver in each evidence of extrahepatic obstruction unrelated to viral hepatitis in and granuloma formation of undetermined origin cholangiocarcinoma and portal cirrhosis in 1 each

Sixty five of the patients with viral hepatitis were male and 13 were female The majority were young adults between the ages of 15 and 30 years The youngest was 1 the oldest was 60 Three of the patients died The age and sex distribution is given in Table 1

TABLE 1
AGE AND SEX OF PATIENTS

Age (yrs)	*Sex* *Males*	*Females*	*Total*	*Per Cent*
10–19	14	2	16†	20 5
20–29	35	5*	40††	51 3
30–39	10	2*	12	15 4
40–49	4	1	5	6 4
50–59	2	2	4	5 1
60–69	0	1	1	1 3
Total	65	13	8	100 0

* 4 pregnant 1 pregnant † 1 fatal case †† fatal cases 1 male 1 female (pregnant)

THE HISTOPATHOLOGIC CHARACTERISTICS OF THE LIVER IN THE DELHI EPIDEMIC OF 1955

The biopsy material in many of the cases exhibited features that varied from the accepted histologic picture of acute nonfatal viral hepatitis in other epidemics throughout the world[1] [3] In fact the differences were sometimes so striking as to throw doubt upon the diagnosis However the clinical characteristics and laboratory findings were entirely compatible with those reported in other epidemics Cultures for complicating microorganisms serologic tests and stains for *Leptospira* were consistently negative Therefore the histologic features seen in biopsies in this epidemic were accepted as representative of the wide variations possible in the histopathological appearance of the liver in viral hepatitis In this discussion the usual changes will be referred to as standard those characterizing many of the Delhi cases as obstructive Histopathologic features in the obstructive type that differ from the standard are as follows

1 Bile stasis in the bile canaliculi is marked even in the earliest stages observed (Fig 19) These usually delicate and almost invisible struc

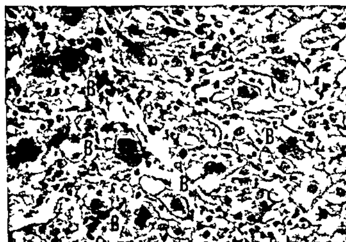


FIGURE 19 Acute nonfatal viral hepatitis with pericholangitic (obstructive type) Liver needle biopsy 10 days after onset of illness Degenerative changes of liver cell focal intralobular necroses with mononuclear cell infiltrate and marked stasis of bile (B) in bile canaliculi (× 330) (AFIP Accession 581362)

tures are often so distended that they appear as glandlike channels with a lumen containing bile and surrounded by liver cells (Fig 20) In some instances portions of lobules are transformed into similar infantile or embryonal bile ductules (Fig 21) The liver cells surrounding the glandlike lumen often show alteration of varying degree particularly vacuolation of the cytoplasm on the side away from the biliary channel This change may sometimes be so extreme as to involve the entire cell

2 Polymorphonuclear leukocytes occur with much greater frequency They are associated with mononuclear cells in the infiltrates in the stroma of the portal areas and in the focal necroses within the lobules (Figs 22 23)

3 Degenerative alterations such as swelling ballooning and vacuolization of individual liver cells are not as prominent acidophilic bodies are uncommon intralobular infiltrates are less frequent lipofuscin in Kupffer cells does not appear as early and is not as distinct as in stand ard lesions but is usually present particularly in the persistent form of the disease (Fig 24)

4 Distortion of the radial columns and the lobular structure as a whole is less conspicuous (Figs 25 26)

No one particular histologic feature can be selected as characteristic of obstructive hepatitis especially since all components associated with the standard picture may also be present However the greater degree of intralobular bile stasis the transformation of the bile canaliculi into embryonal bile ducts and the abundance of polymorphonuclear leukocytes differ significantly from standard features

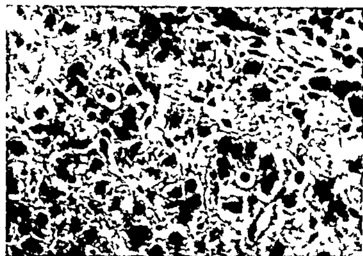


FIGURE 20 Acute nonfatal viral hepatitis, obstructive type Liver needle biopsy 24 days after onset of illness. Transformation of many of the liver cell columns into glandlike structures about the lumen of distended bile canaliculi Droplets of bile in ductules (× 300) (Irwin Hospital No 18/56)



FIGURE 21 Acute nonfatal viral hepatitis obstructive type Liver needle biopsy [illegible]0 days after onset of illness Glandlike arrangement of liver cells about bile canaliculi distended with bile Vacuoles in cytoplasm of liver cells beneath reticulum of sinusoid Many of the Kupffer cells contain argentophilic granules of lipofuscin (K) (Gridley reticulum stain × 450) (Patel Chest Institute No [illegible]/56)

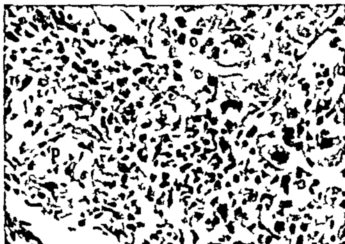


Figure 22 Acute nonfatal viral hepatitis with pericholangitis ("obstructive" type) Liver needle biopsy one week after onset of illness Infiltrates in stroma of portal canal (P) consist of mononuclear cells and many polymorphonuclear leukocytes Extensive degenerative alterations of liver cells and focal necroses in lobules. (× 400) (AFIP Accession 551306)

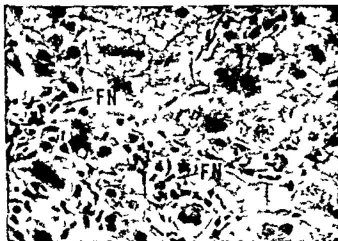


Figure 23 Acute nonfatal viral hepatitis, obstructive type Liver needle biopsy 35 days after onset of illness Degenerative changes of liver cells focal necroses (FN) in lobules with infiltrates consisting of mononuclear cells and polymorphonuclear leukocytes. (× 500) (Hatel Chest Institute No 53/5[illegible])

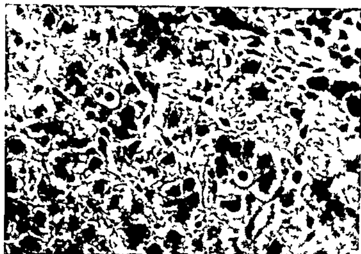


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FIGURE 21 Acute nonfatal viral hepatitis obstructive type Liver needle biopsy 20 days after onset of illness Glandlike arrangement of liver cells about bile canaliculi distended with bile Vacuoles in cytoplasm of liver cells beneath reticulum of sinusoids Many of the Kupffer cells contain argentophilic granules of lipofuscin (K) (Gridley reticulum stain × 450) (Patel Chest Institute No 7/56)

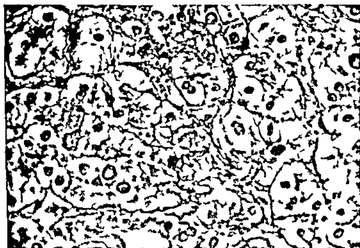


FIGURE 26 Acute nonfatal viral hepatitis "obstructive" type Liver needle biopsy 33 days after onset of illness Degenerative alterations of liver cells with disorganization of cell columns retention of bile in canaliculi formation of glandlike liver cell cords and collapse of stroma with focal condensation of reticulum fibers (Gridley reticulum stain × 450) (Patel Chest Institute No 22/56)

Both the standard and obstructive types of viral hepatitis have various stages referred to as active subsiding (receding or healing) or persisting (subacute or chronic) In the subsiding phase of both types degenerative alteration of liver cells ceases and inflammatory infiltration decreases while regeneration proceeds and dominates the field In the obstructive type bile stasis or its effects may persist for a longer time but the final picture is one of complete restitution of the liver cell columns and restoration of the lobular structure

In the persisting phase there is comparatively little detectable damage to the liver cells but focal accumulations of mononuclear cells within the lobules indicates continuing necrosis of liver cells and the portal mononuclear infiltrates remain visible for quite some time Accomplished liver cell necrosis is recognized by the presence of lipofuscin in Kupffer cells and the focal collapse of the reticulum fibers In addition there are signs of unrest of parenchymal elements in the form of differences in size shape and staining reactions of liver cells which often contain several nuclei In standard types occasional acidophilic bodies may still be seen and in the obstructive type bile stasis although less marked usually persists

Viral hepatitis of obstructive as well as standard type exhibits degrees of severity in its various phases which correspond roughly with similar clinical classifications The histologic types and phases of viral



FIGURE 24. Acute nonfatal viral hepatitis, "obstructive" type. Liver needle biopsy 13 days after onset of illness. Colonies of Kupffer (K) cells containing masses of argentophilic granules of lipofuscin. Degenerative alterations of liver cells and frequent transformation of liver cell columns into "embryonal" bile ductules. (× 300.) (Patel Chest Institute No. 38/56.)

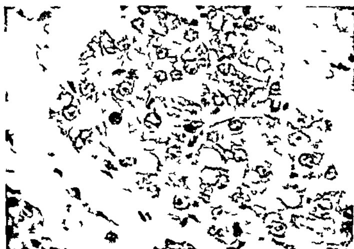


FIGURE 25. Acute nonfatal viral hepatitis, "obstructive" type. Liver needle biopsy 35 days after onset of illness. Extensive disorganization of the radial arrangement by syncytial masses made up of regenerating liver cell columns. (× 400.) (Patel Chest Institute No. 53/56.)

Results of Liver Function Tests of Patients on Whom Liver Biopsy Was Performed

The results of liver function tests are listed in Table 3 where they are compared with the histologic types and phases of viral hepatitis

The results of the few liver function tests performed on patients with viral hepatitis of several types and phases show that the highest values for serum bilirubin thymol turbidity flocculation and serum phosphatase were obtained during the persisting phase of one case of the obstructive type The serum bilirubin values were comparable in the standard and obstructive types while thymol turbidity and flocculation were slightly greater in cases of the standard type a possible expression of more extensive parenchymal damage As expected the lowest values were found in the subsiding phases The present series is entirely too small for the drawing of significant conclusions concerning a relation between the results of liver function tests and the types and phases of hepatitis but it gives a general impression that some correlation may exist between the severity of the illness and the outcome of the tests

Table 3

AVERAGES OF RESULTS OF LIVER FUNCTION TESTS IN DIFFERENT TYPES AND PHASES OF HEPATITIS

Histolog Type or Phase of Hepatitis	*No of Cases*	*Bilirubin*				*Thymol*		*Alk Phosphatase#*
		Dir	*Ind*	*Tot*	$\frac{d}{T} \times 100$	*Turb*	*Floc*	
Standard	5	3.4	2.0	5.4	60.3	10	3+	Not determ
Obstructive	15	3.5	1.9	5.4	63.4	7	2+	33
Subsiding*	8	1.5	1.[illegible]	2.7	58.1	6	2+	9‡
Persisting	1	10.0	5.0	15.0	66.6	10	3+	4[illegible]
Total	29							

K A units * 7 standard cases 1 obstructive case [illegible] obstructive case ‡ standard case

Viral Hepatitis and Pregnancy

The susceptibility of pregnant women to viral hepatitis and the grave danger to which this disease exposes expectant mothers have been pointed out in previous epidemics [47] Accurate information on this point was not available for the Delhi outbreak but the few data we obtained from 5 women in our series whose pregnancy was complicated by viral hepatitis are summarized in Table 4

Viral hepatitis was of the obstructive type in these 5 cases The only babies who survived were delivered at full term before the clinical onset

TABLE 2

TYPES, PHASES AND DEGREES OF SEVERITY OF LESIONS IN VIRAL HEPATITIS

	Type						
	Standard			*Obstructive*			
Degree of Severity	*Active*	*Subsiding*	*Persisting*	*Active*	*Subsiding*	*Persisting*	*Total*
Mild	4	4	0	0	0	0	8
Mod severe	14	8	0	28	7	4	61
Severe	1	0	0	5	0	0	6
Fatal	2*	0	0	1	0	0	3
Total	21	12	0	34	7	4	78

* Type uncertain

hepatitis encountered in the biopsies of the 78 cases are enumerated in Table

The degree of severity of the infection was established on the basis of the following changes in parenchymal cells (necrosis swelling ballooning vacuolation number of acidophilic bodies evidence of regeneration) the amount of lipofuscin in Kupffer cells (Fig 4) (expressing the degree of destruction of liver cells) the amount of bile stasis in bile canaliculi (Fig 19) rupture of canaliculi and exudation of bile into tissues as well as the presence of bile particles in liver and Kupffer cells the extent of intralobular infiltrates composed of mononuclear cells and polymorphonuclear leukocytes (Fig 3) and of portal infiltrates which also include eosinophils (Fig) the number of intralobular focal necroses (Fig 3) distortion of liver cell columns collapse and condensation of argentophilic reticulum fibers (Fig 6) general disturbance of the lobular architecture (Fig 5) scarring and state of preservation of blood vessels and bile ducts

Active cases of the obstructive type were most common active cases of the standard variety were next in number The clinical data indicate that the subsiding and persistent infection usually occurred in the relatively older age group while the fatal fulminating variety was in younger patients Since follow up observations are practically nonexistent nothing can be said concerning the total duration or the outcome of the disease in most of the cases However the few repeat biopsies on standard as well as on obstructive types indicate that healing occurs in both

veins the stroma of the portal areas contained an unusual and significantly greater number of polymorphonuclear leukocytes in conjunction with mononuclear cells Because of the resemblance of these features to those of pericholangitis the disease in this outbreak was tentatively referred to as the pericholangitic form of viral hepatitis Glandular transformation of the bile canaliculi was not observed in these cases or in those of a series described by Gall and Braunstein[17] in which clinical symptoms of bile duct obstruction were associated with histologic changes rather closely resembling those observed in the Texas outbreak An additional feature noted by Gall and Braunstein was pseudoductular budding in and about the portal areas

Glandular transformation of the bile canaliculi appears to be related to stasis and retention of bile The histopathologic relationship of the obstructive type of hepatitis to the cholangiolitic type of Watson and Hoffbauer[76] is not clear Glandular bile ductules observed in cases of neonatal jaundice of unknown origin have been thought to represent embryonal bile ducts[69] The transformation of liver cells into ductlike structures in the neonatal disease is much more marked and generalized than in viral hepatitis of the obstructive type

Such atypical changes have so seldom been encountered in viral hepatitis that their pathogenesis is still in question Are they due to a modified virus to conditions complicating the usual effect of the virus on the liver tissue or to a modified host virus relationship? It has been suggested that the nutritional state of the host may influence the effort of the virus and thus that the widespread border line malnutrition in India may be a factor in the production of atypical forms of the disease However the hepatitis in the Agra epidemic was of the standard type and in the six years that elapsed before the outbreak of the Delhi epidemic the state of nutrition of the Indian population has improved A more tenable view is that differences may exist between the viruses causing epidemics or sporadic cases of hepatitis in different parts of the world or even in the same locality Proof of this might be obtained from immunologic studies similar to those used in distinguishing between the viruses of infectious and homologous serum hepatitis wherein reciprocal tests were conducted on human volunteers[3, 4, 5, 7]

As regards the obstructive component the presence of polymorphonuclear leukocytes in the stroma of the portal canals may have the same significance as it does in pericholangitis In both conditions there is retention of bile in bile canaliculi especially in the centrolobular zone in both the clinical manifestations of obstructive jaundice are prominent in the absence of surgical obstruction of the bile passages. Since the mononuclear infiltrates in the portal areas despite their density apparently do not cause biliary obstruction or bile retention in the standard type the

TABLE 4

VIRAL HEPATITIS AND PREGNANCY

No	Age of Patient (yrs)	Duration of Pregnancy (mos)	Type of Hepatitis	Duration of Disease (days)	Fate of Fetus	Comments
1	20	5	Obstructive*	5	Died undelivered	No autopsy
2	30	9	Mod severe obstructive	9	Normal baby*	No follow up
3	[illegible]	9	Mod severe obstructive	14	Normal baby *	No follow up
4	29	8	Severe obstructive	14	Premature died†	Autopsy normal
5	28	7	Severe obstructive	15	Premature died††	No autopsy

Fatal case of mother
D livered 5 days prior to biopsy
D livered 15 days prior to biopsy
† Died 4 days after delivery
†† Died 3 days after delivery

of hepatitis and according to hospital records they were normal At autopsy of one of the premature babies (No 4 of Table 4) who died 4 days after delivery no significant pathologic changes were seen in the liver A fetus of 5 months gestation died undelivered *in utero* when hepatitis was fatal to the mother the 7 month baby who died 3 days after birth had no clinical evidence of jaundice and autopsy was not performed

INTERPRETATION OF THE HISTOPATHOLOGIC FEATURES IN LIVER NEEDLE BIOPSIES DURING THE DELHI EPIDEMIC OF VIRAL HEPATITIS

For a satisfactory assessment of the significance of the atypical histologic features in many of the cases of the Delhi epidemic the type of viral hepatitis usually encountered in India should be known The only available information on this point was gathered in the epidemic of viral hepatitis in Agra in 1955 According to Wahi the histopathologic findings in biopsies performed during this epidemic were consistent with those here referred to as standard No histopathologic studies were carried out during the outbreak of viral hepatitis in Ceylon, which involved army personnel in World War II [3]

Histopathologic features somewhat similar to those that characterized the disease in the majority of cases of the Delhi epidemic had previously been reported in a group of patients from Brooke Army Hospital San Antonio Texas U S A [16] Bile retention even in the early stages of hepatitis was particularly prominent in the bile canaliculi about the efferent

cirrhosis or giant cell transformation of liver cells has not been presented

Progression from clinical manifestations and pathologic alterations characteristic of viral hepatitis to cirrhosis of either the portal or the post necrotic variety has not been observed in the wake of the Delhi epidemic In several instances postnecrotic scarring has been demonstrated In a few cases of both types in which liver function tests indicated clinical recovery repeat biopsies have shown that the parenchyma was regaining its normal appearance even after extensive damage In two cases of the obstructive type biopsies performed 60 and 180 days after onset demonstrated persistent viral hepatitis Further follow up information when available may reveal other sequelae of viral hepatitis as seen in this epidemic

Summary

During the Delhi epidemic needle biopsy of liver confirmed the clinical diagnosis in 78 of 90 patients

Forty five biopsies presented an unusual variety of viral hepatitis to which the term obstructive type was applied Thirty three exhibited those lesions accepted as the usual or standard type The several stages of the disease were represented in both types

The obstructive type of viral hepatitis has hitherto rarely been observed It is characterized by a peculiar transformation of bile capillaries into glandlike structures or embryonal bile ductules composed of radially arranged liver cells around the lumen of a bile canaliculus which is distended with bile Polymorphonuclear leukocytes are prominent in the portal infiltrates

What causes the histologic appearance of the obstructive type to deviate from the standard is not known but the two types may be seen in the same epidemic as in Delhi

Of five needle biopsies of liver from pregnant women with viral hepatitis all exhibited changes of the obstructive type Three premature infants born of mothers with viral hepatitis died autopsy performed on only one showed no evidence of hepatitis

Clinically viral hepatitis of the obstructive type does not differ from that of the standard type All stages from mild nonfatal to fulminant were observed in both types during the Delhi epidemic Three patients died of acute yellow atrophy In the majority of the remaining patients recovery proceeded smoothly with restoration of normal liver function

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association of polymorphonuclear leukocytes with bile stasis may be significant At any rate the same factors that are at work in producing intrahepatic biliary obstruction in simple pericholangitis are presumably active in this atypical hepatitis

It is interesting to speculate why similar values of bilirubin were found in standard and obstructive cases In the former in which histologic evidence of obstruction of biliary passages or bile retention is lacking the more extensive cellular damage may permit rupture of the bile canaliculi and escape of bile into the lymphatics and sinusoids The very fact that in the obstructive type the glandlike canaliculi become distended with bile but still do not rupture indicates that the capillary biliary passages maintain a certain integrity this is corroborated by histologic evidence that the parenchymal cells are less severely damaged in this variant of hepatitis

Since actual obstruction of the lumina of bile ducts has not been observed it is probable that the free flow of bile is impeded by an over all reduction of the capacity of the biliary passages within Glisson s capsule by edema and by the acute inflammatory reaction Whether this reaction is caused by an atypical virus or other complicating microorganisms is not known Laboratory studies conducted on the group of cases in the Texas epidemic [16] have not as yet produced results that would explain this point

Whatever causes the difference between them both types of hepatitis eventually subside While the presence of both types side by side in the same epidemic cannot be explained it does provide a problem in geographic pathology

The greater gravity of viral hepatitis during pregnancy has been observed in previous epidemics the Delhi epidemic was no exception and circumstances justified the distribution of the limited supply of gamma globulin [1, 6, 7] to pregnant women Statistical evaluation of the prophylactic results of this medication is not yet available

In a few instances the virus causing hepatitis in the mother has apparently been transmitted to the fetus [74] Whether this has ever caused unequivocal viral hepatitis of the fetus *in utero* or only viremia will remain controversial until the histopathologic criteria of viral hepatitis in the fetus can be established The many possible causes for neonatal jaundice must be thoroughly explored before viral hepatitis can be held responsible for the entire gamut of neonatal and postnatal hepatic disease The assumption that viral hepatitis causes infantile cirrhosis [1, 8] is rather inadequately supported by clinical and pathologic data [87] This applies also to fetal viral hepatitis as a cause of neonatal transformation of liver cells into giant cells [14, 70] Although the possible influence of viral infection on the fetus *in utero* should not be ignored irrefutable proof of a definite relation between transmission of the virus *in utero* [47] and fetal hepatitis infantile

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6

Pathologic Aspects of the Late Stages of Viral Hepatitis

ARCHIE H BAGGENSTOSS, M D
(Rochester Minnesota)

This report is concerned primarily with instances of viral hepatitis in which hepatic damage was neither so mild that complete restoration of the hepatic parenchyma took place nor so severe that a rapidly fatal outcome resulted It is concerned then with patients who lived long enough for some degree of hepatic regeneration to occur but in whom the injury was so severe or so often repeated that anatomic restoration of a normal liver could not take place

No investigator can be certain in a study of this kind that a virus was the etiologic factor in every case since methods for proving the presence of the virus are not available For purposes of this report only those cases were used in which the clinical data strongly suggested that viral hepatitis had been present No case was included in which there had been a history of operation on the biliary ducts exposure to hepatotoxic chemicals cardiac failure thyrotoxicosis hemolytic anemia alcoholism or other complicating conditions Information at necropsy automatically excluded those cases in which extrahepatic obstructive jaundice was present The majority of the patients included in this study had an illness characterized by the onset of jaundice associated with anorexia nausea and ill defined abdominal distress This paper does not deal with any single series of cases but presents some previously published data as well as more recent observations based on various material studied by my associates and me in the Section of Pathologic Anatomy at the Mayo Clinic

General Hepatic Changes in Viral Hepatitis

The variety of lesions that one may observe in the intermediate zone of hepatic damage by viral hepatitis is truly amazing At one end of the spectrum is the almost total destruction of hepatic parenchyma with minimal regeneration so well described by Lucke [1] Lucke and Mallory [2] and Werthemann [3] At the other end is almost total parenchymal restoration by nodular regeneration Because of the remarkable regenerative power of the liver any lesion produced during viral infection is never

(Figure 5c) We are dealing then with the three processes fundamental to the definition of cirrhosis [6] It is my contention that if the hepatic damage were somewhat less and the time of survival somewhat longer the regeneration would be more extensive and nodular and a hepatic lesion would evolve that anyone would designate as cirrhosis [3, 7-16]

The histologic aspects of these hepatic lesions will not be considered further at this time since they have been adequately described by others [3] This much has been mentioned here in order to emphasize that when we deal with the pathologic anatomy of the late stages of viral hepatitis we are led inexorably to the problem of posthepatitic cirrhosis

General Aspects of Posthepatitic Cirrhosis

One cannot draw a sharp line of distinction between the lesions just discussed (subchronic atrophy subacute diffuse necrosis or postnecrotic scarring) and so called posthepatitic cirrhosis Some of the former lesions merge almost imperceptibly into the latter condition For the purposes of this study however posthepatitic cirrhosis includes only those cases in which regeneration was predominantly nodular that is the regenerative process did not produce lobules with normal vascular relationships but nodules without central veins or with veins that were extremely eccentric [6]

The question as to the frequency with which cirrhosis develops after single or repeated attacks of viral hepatitis never has been answered satisfactorily Studies by Zieve and co workers [16] suggested that the true incidence of cirrhosis following acute infectious hepatitis may be considerably less than was considered heretofore However the possibility that viral hepatitis may be more important as a cause of cirrhosis than has been realized previously was suggested by Real Encinas [17] who reviewed all the cases of cirrhosis observed at necropsy for a 10-year period at the Mayo Clinic He found that 37 of 157 cases (4 per cent) could be classified as posthepatitic in origin on the basis of the history and the pathologic examination Another possible clue to the importance of hepatitis as a factor in cirrhosis was noted by Craig and associates [18] who found that 30 of 37 cases (81 per cent) of cirrhosis in children exclusive of obstructive cirrhosis were associated with hepatitis Ruggieri [19] reviewed the cases of juvenile cirrhosis exclusive of obstructive biliary cirrhosis seen at the clinic and noted that 16 of 27 patients (59 per cent) had histories of a disease process with clinical manifestations of viral hepatitis as a precursor of the cirrhosis Also the clinical histories in the remaining 11 cases did not exclude preceding hepatitis but merely did not contain sufficient data to justify such a diagnosis In all but one of these cases of juvenile cirrhosis the liver had the gross and histologic appearance of postnecrotic or toxic cirrhosis — terms that signify a morphologic type

static but immediately is subject to alteration by parenchymal regeneration It is the manifestations of the variations in extent distribution and intensity of this regenerative power that are largely responsible for the variety of lesions encountered The greatest degree of destruction often occurs near the hilus and extends out toward the periphery in a fan shaped manner with regeneration most prominent at the edges of both lobes (Figure 1a) In other cases regeneration is practically absent in the left lobe and diffusely distributed throughout the remainder or is localized to a portion of the liver (Figure 1b)

In all these cases destruction of the parenchyma is the outstanding feature and these lesions commonly are called subchronic atrophy post necrotic collapse or postnecrotic scarring of the liver[4, 5] In addition to parenchymal destruction however histologic examination gives evidence of parenchymal regeneration of variable degree and a relative increase in connective tissue albeit the latter consists largely of collapsed stroma

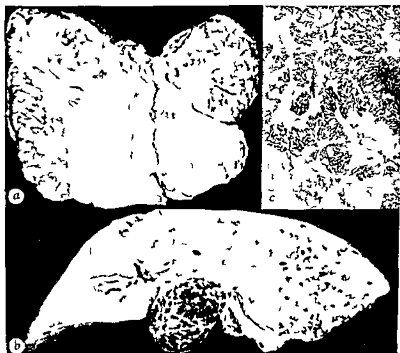


FIGURE 1 Subchronic atrophy of the liver in viral hepatitis *a* Note zone of atrophy extending out from hilus with regeneration at periphery (weight 945 gm) *b* Note atrophy of left lobe with regeneration in right and quadrate lobes (weight 1387 gm) Early regeneration Note how contiguous portions of lobules have survived to form unusual patterns (Hematoxylin and eosin × 17)

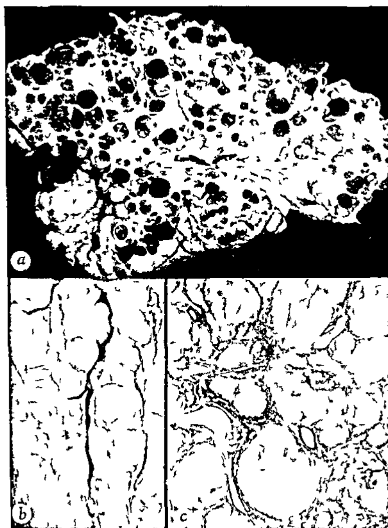


FIGURE 3 Posthepatitic cirrhosis nodular type *a* Note compression of veins *b* Note distortion of vein by regenerative nodules *c* Note compression of veins by regenerative nodule (Elastin H Van Gieson × 6)

nodules were not so large and were distributed more diffusely throughout the liver (Figure 3) Although the nodules were separated by zones of atrophy these were not as broad and extensive as were those in the lobar group The diameter of the nodules varied from 5 mm to cm or more They were generally yellow or green and the intervening atrophic zones

of cirrhosis which is the commonest variety caused by viral hepatitis Also suggestive of the importance of hepatitis was a recent survey by Stuhler and associates [9] of all the cases of cirrhosis in women exclusive of obstructive biliary cirrhosis seen at the clinic They studied necropsy data in 90 cases encountered over a period of 31 years This group included 75 cases (83 per cent) classified as postnecrotic cirrhosis and only 15 (17 per cent) classified as Laennec's cirrhosis There was a history of hepatitis in 39 of the 75 cases (5 per cent) of postnecrotic cirrhosis

CROSS APPEARANCE IN POSTHEPATITIC CIRRHOSIS

On the basis of macroscopic appearance Stauffer and I [1] classified our cases of posthepatitic cirrhosis into three groups lobar nodular and granular

In the lobar group the nodules of regeneration were extremely large the normal contour of the liver was destroyed by the huge isolated nodules some of which were the size of hepatic lobes (Figure) These large regenerative nodules or lobes were separated from each other by deep indentations or by broad zones of atrophy They were located usually at the periphery of the right or left lobes or were limited almost entirely to the right lobe The livers in this group were reduced greatly in size and weight

In the second group which included most of the cases the regenerative



FIGURE Posthepatitic cirrhosis lobar type Note atrophy of left lobe (weight 710 gm)

were wider than normal and contained increased numbers of lymphocytes, phagocytes, bile ducts and connective tissue fibers. The regions around the central veins often showed evidence of passive congestion and atrophy. In other instances, however, the portal tracts appeared extremely small, with diminutive blood vessels and tiny bile ducts surrounded by an exceedingly scanty fibrous stroma, as pointed out by Smetana. Also, these hepatic lobules frequently appeared to be much larger than normal, with wide stretches of hepatic parenchyma separating the afferent and efferent vessels. It is difficult to determine whether these changes are the result of reorganization and reorientation of regenerated hepatic tissue after necrosis and destruction of large portions of hepatic parenchyma, or whether these are islands of hepatic parenchyma that have survived the original injury and have undergone intralobular regeneration.[23, 24] Both types of regeneration undoubtedly occur.

In other large nodules, however, the architectural pattern was altogether different in that the nodule was made up of composite groups of hepatic cells in which the normal relationship between portal tracts and central veins was lost (Figure 5b). In these groups of proliferating cells the central vein often could not be located or, if found, was eccentric and in close proximity to the portal tracts. These groups of regenerating hepatic cells, which, when found in large aggregates, made up some of the large nodules of the lobar and nodular groups of livers, also were observed as small solitary collections in the granular livers (Figure 5c). Because they fail to restore the normal architectural pattern, they no longer can be designated as lobules but, perhaps, are better designated as regenerative nodules.

Depending on the extent of the destructive process, the location and number of surviving groups of cells and the vigor of the regenerative phase, there is generally great variation in the size of the regenerative nodules. As already indicated, some of them probably contain whole groups or remnants of former lobules. If sufficient time has elapsed after the injury, they are generally oval or spherical, but, depending on the location of the destructive process within the lobule and its relation to necrosis of cells in neighboring lobules, the regenerative process may lead to the development of garland-shaped or other bizarre forms[24] (Figure 5d).

The regenerative nodules in many of the livers were not completely formed and, from evidence of cellular regeneration, appeared to be actively enlarging. Individual hepatic cells were frequently multinucleated and often they were unusually large and contained bizarre hyperchromatic and giant nuclei. The cellular proliferation produced laminae that were two cells or more in thickness, and was accompanied by the production of a new reticulum and sinusoidal framework that did not have

were usually red or gray The regenerative nodules in both this and the former group revealed many instances of gross distortion of the hepatic and portal veins by small and large nodules (Figure 3) The venous distortion was most severe in those cases in which the regenerative nodules were most numerous and close together Thus venous distortion was greatest when regenerative activity had been most vigorous These livers also were reduced greatly in size and weight

In the third group of cases the livers were finely nodular or granular The regenerative nodules in these cases were small the greatest diameter was generally 0 5 cm or less The nodules in some cases were separated widely by broad atrophic zones and in others were close to one another with correspondingly narrow atrophic zones (Figure 4)

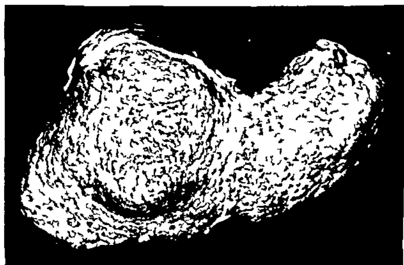


FIGURE 4 Posthepatitic cirrhosis granular type

HISTOLOGIC APPEARANCE IN POSTHEPATITIC CIRRHOSIS

Lobar and Nodular Types Sections taken from the regions of nodular regeneration varied considerably in architectural pattern and in the relation of the afferent and efferent veins to the hepatic parenchyma In sections taken from some of the large nodules of regeneration observed in the lobar and nodular types the lobular pattern often was partially preserved insofar as the relationship of the central veins and portal tracts to each other and the hepatic parenchyma was concerned (Figure 5a) These large nodules contained many central veins and portal tracts indicating that they were multilobular in origin The portal tracts in some instances

the usual orientation to the central vein The old framework of sinusoids and reticulin fibers had collapsed and was shoved peripherally by the new centrifugal growth of hepatic cells

By a process of condensation the old framework became scar tissue According to this concept the fibrous tissue in cirrhosis represents to a large extent the accumulated skeletons of previously destroyed lobules that have been pushed aside by the vigorous regeneration of hepatic cells Because of the eccentricity of the position and proliferation of these hepatic cells the central vein comes in time to occupy a position toward the periphery of this new nodule or pseudolobule

Granular Type In about half of the granular livers the regenerative nodules appeared to have achieved as nearly complete development as possible within the bounds of the surrounding collapsed stroma (Figure 6a) A certain balance apparently had been achieved between the dynamic expanding growth of the nodule and the passive yielding but ultimately compressed stroma that represented the collapsed framework ducts and vessels of previously destroyed lobules or portions of lobules According to Nunes [20] the restoration of the limiting membrane between the nodule and the compressed stroma is of great importance in determining whether the continuity of the cholangioles with the interlobular ducts is restored Consequently the restoration of the limiting membrane is of considerable significance in determining whether jaundice occurs at this stage The gross and histologic features in these granular livers were identical with those observed in so called alcoholic or Laennec s cirrhosis

In the remaining livers of the granular group the hepatitis was still active with continuing destruction of hepatic cells and relatively broad zones of atrophy between tiny and incompletely developed regenerative nodules (Figure 6b) Inflammation of veins was generally present whereas infiltration with fat was mild or absent Consequently although these livers were grossly granular they did not present the histologic characteristics of alcoholic cirrhosis Transitions of hepatitis to a type of cirrhosis simulating primary biliary cirrhosis [27] (Hanot s cirrhosis) or cholangiolitic cirrhosis [11] also have been described but were not apparent in this material A study of the cases of primary biliary cirrhosis seen at the clinic is now in progress

The present report likewise contains no observations on the so called chronic hepatitis syndrome Studies at the Armed Forces Institute of Pathology with many serial observations reveal that the basic pathologic changes in this syndrome are essentially identical to those in acute non fatal hepatitis except that they are more focal and less extensive

Changes in Stroma The atrophic internodular regions presented a variable

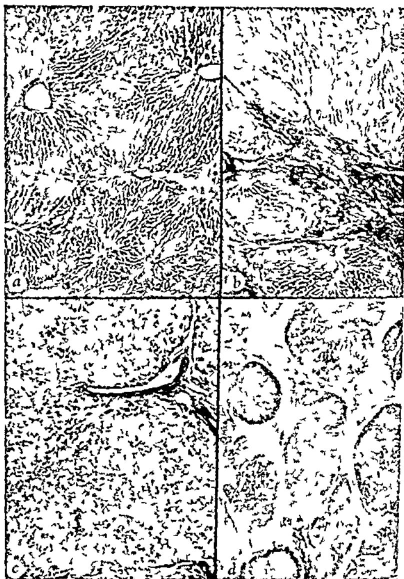


FIGURE 5 Posthepatitic cirrhosis *a* Section of a large nodule Note that the relationship between the portal vein and the central vein is normal in many lobules Also note evidence of passive congestion (Hematoxylin and eosin × 23) *b* Section from a large nodule in which some regenerative nodules no longer have central veins (Elastin H Van Gieson × 23) *c* Granular type Note compression of vein by nodules (Hematoxylin and eosin × 54) *d* Regenerative nodules of unusual shape (Hematoxylin and eosin × 15)

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Changes in Stroma The atrophic internodular regions presented a variable



FIGURE 6 Posthepatitic cirrhosis granular type *a* Regenerative nodules are small and well demarcated with narrow internodular zones as in Laennec's cirrhosis *b* Regenerative nodules are incompletely developed with hepatitis still active and broad internodular zones of atrophy (Both hematoxylin and eosin × 65)

histologic appearance depending to a large extent on the lapse of time since the acute attack In disease of relatively short duration sections of the internodular zones revealed large numbers of bile ducts and ductules lymphocytes and plasma cells and smaller numbers of polymorphonuclear cells in addition to vessels Branches of the portal or hepatic veins often revealed evidence of mild inflammation as indicated by the presence of

lymphocytes in their wall Most of the connective tissue in these atrophic internodular zones consisted of the remnants of the collapsed reticulum of previously destroyed lobules Formation of new connective tissue as indicated by the presence of fibroblasts and collagenous fibrils appeared to be minimal in most of these livers In disease of longer duration there was evidence of compression of the internodular stroma by the growth of the regenerative nodules and transformation of some reticulin fibers into thicker collagenous fibrils appeared to have taken place

The internodular connective tissue and exudate often appeared to extend into the regenerative nodules a picture that often has been interpreted as invasion of the parenchyma It is my opinion that such invasion of regenerated parenchyma does not occur rather the appearance of connective tissue septa always is preceded by destruction of hepatic cells with collapse of supporting stroma and secondary exudative reactions

Changes in Blood Vessels Of particular interest in this dynamic process of destruction and repair is the fate of the pre existing vessels Attention already has been called to the gross distortion of the portal and hepatic venous radicles produced by the nodules of regeneration The same process can be demonstrated histologically (See Figures 3c and 5c) In order to study this process in greater detail Kelty and co workers [4] made a glass plate reconstruction of serial sections taken from a liver that was the site of postnecrotic cirrhosis From this glass plate reconstruction it was possible to make a wax model that clearly demonstrated the compression and distortion of radicles of the hepatic veins and related the compression not to fibrous connective tissue but to growth of the regenerative nodule This work has been confirmed by Popper and associates [?] Another method namely the vascular injection of vinyl acetate and subsequent corrosion of the injected specimen was used at the clinic by Mann and co workers [30] and it also illustrated the distortion of the vascular bed by the regenerative nodules The expanding growth of the regenerative nodule compresses the surrounding vessels into a basketlike network It was not possible by this method to demonstrate shunts between radicles of the hepatic artery and vein However shunts between the portal vein and the hepatic veins were relatively easy to demonstrate and emphasized the fact that the regenerative nodules are at a definite circulatory disadvantage with regard to perfusion by portal blood This may be a partial explanation of the frequently seen ischemic necrosis in regenerative nodules The imperfect blood supply of the regenerative nodule also may explain in part the progressive nature of cirrhosis and the functional failure that occasionally develops long after the viral infection itself has disappeared

The lesions of postnecrotic or multilobular cirrhosis provided ideal ma

terial for the demonstration that distortion of vessels unquestionably occurs in this variety of cirrhosis (See Figures 3 and 5c) The principle illustrated by this phenomenon has a much broader application however Our observations suggested that the same distortion which we demonstrated in vessels large enough to follow in serial section reconstructions and in vascular injection and corrosion methods also occurs in each tiny central vein in alcoholic cirrhosis It is probable from the hemodynamic point of view that the compression of the sinusoidal bed and the distortion of the myriads of central veins which occur when regenerative nodules replace destroyed lobules are of much greater importance in the pathogenesis of portal hypertension than is the distortion of larger vessels This destruction and distortion of the vascular tree at the very termination and at the very beginning of the hepatic portal and hepatic venous systems respectively are of greater significance in the development of portal hypertension In our experience the anatomic basis for interference with the portal circulation does not lie in any constricting effect of connective tissue or in the production of arteriovenous shunts but in the tremendous destruction of the original capillary bed and in the severe distortion of vessels and vascular relationships brought about by the regenerative nodules which is a manifestation of the great regenerative powers of the liver According to this concept growth of the regenerative nodule distorts the course of the central vein and compresses the collapsed sinusoidal bed contributing to the obstruction to portal flow

Effects of Cirrhosis on Hepatic Lymphatic Vessels

In addition to studying the effects of posthepatitic cirrhosis on the capillary and venous bed and its relation to portal hypertension my associates and I also have been concerned with the effects of cirrhosis on the lymphatic vessels particularly in relation to the presence or absence of ascites It is notoriously difficult to study or even locate the lymphatic vessels in either normal or diseased livers It has been shown however that the deep lymphatic vessels of the liver follow the course of the portal veins and bile ducts and drain toward the hilus of the liver[31–33] and it is considered generally that the predominant flow of lymph from the liver is in this direction

Consequently Cain and I[34] *considered that study of the lymphatics* at the hilus of the liver might give some information regarding disturbances of flow of lymph within this organ We were interested especially in the number size and appearance of the lymphatic vessels in various pathologic states particularly those in which ascites is an associated feature The specimens were obtained at necropsy as soon as possible after death Long bladed clamps were placed across the hepatoduodenal ligament at two locations one as close as possible to the duodenum and the

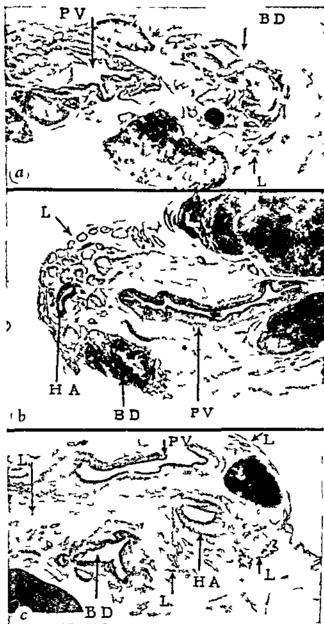
other as close as possible to the hilus of the liver This trapped lymph in the vessels The ligament then was cut beyond the clamps and the specimen with the clamps was immersed in formalin for fixation Later sections were cut and stained with hematoxylin and eosin and by the elastin H Van Gieson method Only sections that included the hepatic artery portal vein and bile duct were studied

In order to obtain an idea as to the variations to be expected in number and size of lymphatic vessels specimens were studied in 100 consecutive routine necropsies in which ascites was absent The number of lymphatic vessels at the hilus in this series varied from 6 to 87 with a mean of 34 Thus in a series such as this in which practically all patients have had diseases of varying types and durations it is difficult to come to any conclusion as to the normal number and appearance of the lymphatic vessels

A fairly good idea of the normal perhaps can be obtained from observations in two cases in which death occurred accidentally and in which disease processes were not observed during life or at necropsy A 33 year old man died of cerebral hemorrhage shortly after a blow on the head during a fight and a 54 year old woman drowned The lymphatic vessels in both cases were small and numbered 19 and 33 respectively (Figure 7a) It is interesting to compare these with obviously abnormal lymphatic vessels in a case of subacute viral hepatitis 75 lymphatic vessels were present and they were remarkably dilated containing large numbers of inflammatory cells in the lumina (Figure 7b)

We studied the lymphatic vessels in 9 cases of posthepatitic cirrhosis with *ascites The mean number of lymphatic vessels was 59* The lymphatic vessels in a case of posthepatitic cirrhosis are illustrated in Figure 7c Dilatation and hypertrophy of the walls of the lymphatic vessels are apparent The findings were essentially similar in alcoholic cirrhosis with ascites In 10 cases of cirrhosis without ascites however the lymphatic vessels also were greatly increased in number and possessed thickened walls

The significance of these observations is not yet clear and the studies are being extended However it appears that the lymphatic vessels draining the liver are greatly increased in size and number in both viral hepatitis and cirrhosis and the increase in the latter probably occurs before ascites appears The additional vessels probably represent the opening up of potential channels that were not seen in the control sections at the magnification used Their presence probably reflects a great increase in the formation *of hepatic lymph in these conditions thus corroborating exper*imental studies on hepatic lymph in injury and cirrhosis of the liver in animals[3] The increased lymphatic drainage in viral hepatitis probably results from destruction of tissue and sinusoidal damage venous stasis and portal hypertension[30] probably also play a part in posthepatitic



PV po t l   BD b le d ct   L lymph t   l   HA h p t rt y

FIGURE 7 *a* The lymphatic ves els number ng 9 are small in the hepato duodenal ligament in a 33 year ol l man h d e l f a head injury *b* The lymphatic vessel numl ering 75 are dil t d n the l epato lu d n l ligament in a case f subacute viral hepit ti witl a ite The lympl atic vessels numbering 6(are dilated in the hepat duod nal ligament in a case of post hepatitic cirrhosis with ascites (All hematoxylin and eos n × 3)

cirrhosis It may be that thickening of the walls is simply a response to increases in flow and pressure within the lymphatic channels over a long period which is a phenomenon analogous to the hypertrophy of arterial walls that occurs in arterial hypertension

Summary and Conclusions

Viral hepatitis occasionally may damage the liver to such an extent that complete restoration of the pre existing structure is not possible When death does not occur during the acute phase variable amounts of parenchymal regeneration take place When the remnants of viable hepatic parenchyma are of variable size and haphazard distribution the regenerative process leads to the development of nodules of variable size shape and location (multilobular postnecrotic toxic cirrhosis) When the damage is more diffuse but does not completely destroy entire lobules over large regions the regeneration likewise is more diffuse and leads to the development of smaller regenerative nodules Depending on the degree of destruction and the vigor of regeneration these small nodules may be separated by broad or narrow bands of atrophy and fibrosis When the regenerative nodules are small and the bands of internodular atrophy and fibrosis are narrow the gross and histologic structure resembles that of alcoholic or Laennec s, cirrhosis

Because regeneration under these conditions is nodular rather than intralobular the normal vascular arrangement is altered profoundly with severe compression and distortion of the small veins and the capillary bed This leads to interference with both portal and hepatic venous flow which probably is an important factor in the development of portal hypertension The development of intrahepatic shunts between the portal vein and the hepatic veins places the regenerative nodule in a precarious position with regard to adequate circulation This may be a factor in the relatively common finding of ischemic necrosis in these nodules and may contribute to the progression of the cirrhosis in the absence of further viral infection

Both viral hepatitis and posthepatitic cirrhosis are associated with a pronounced increase in the size and number of lymphatic vessels draining the liver In cirrhosis this increase apparently occurs before ascites ap pears Destruction of tissue and capillary damage in viral hepatitis are probably responsible for increased formation of lymph whereas venous stasis and portal hypertension probably are additional factors of impor tance in posthepatitic cirrhosis

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7

Cirrhosis in Young Females Its Possible Relation to Infectious Hepatitis

H G KUNKEL M D and A G BEARN
(New York New York)

The etiological factors involved in cirrhosis of the liver in young adults represent one of the most perplexing problems in the field of hepatic disease The virus of infectious hepatitis is certainly the responsible agent in some cases and perhaps also in the great majority However the lack of specific tests for this disease has made it impossible to clearly implicate such an agent in many individuals

During the past 10 years we have observed 37 patients with nonalcoholic cirrhosis of the liver between the ages of 10 and 30 In a large proportion of this group the causative agents could not be determined A breakdown of these patients indicated that were finally found to have Wilson s disease 3 Chiari s syndrome 6 cirrhosis after clear attacks of infectious hepatitis 7 cirrhosis after possible hepatitis and 19 cirrhosis of unknown etiology Thus approximately one half of the patients in this age group had a cirrhosis of obscure etiology

A special study of these 19 patients revealed that they possessed a number of unusual features in common Sixteen of the 19 patients or 84 per cent were females Figure 1 illustrates 3 of these patients at an early stage of the disease The onset of the illness was usually insidious and the average age was 15 Delayed menstruation or amenorrhea was one of the earliest complaints in the majority of these patients Acne of the face neck and shoulders striae and obesity were often observed Arthritis ranging from mild reddening and swelling of interphalangeal joints to a crippling disease involving all extremities was found in approximately one third of the group

Laboratory investigation revealed unusually high total protein levels in the serum with 9 of the patients showing levels above 9 5 gm per cent at some stage of their illness Electrophoretic analyses showed that this increase was almost entirely due to elevation of the gamma globulin fraction Figure 2 *shows the extreme abnormality found in one case where* the total protein was 11 gm per cent (middle patient depicted in Figure 1) This pattern taken early in the disease in many ways resembles that

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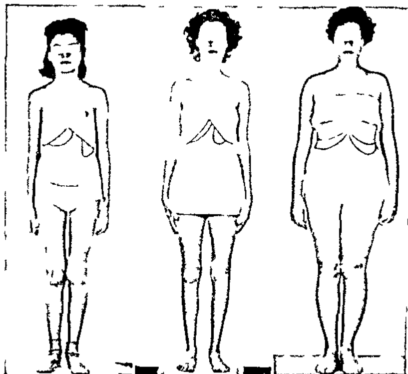


Figure 1 Three female patients with cirrhosis of undetermined etiology Photograph taken during the early stages of the disease

seen in patients with multiple myeloma In most of the cases a partial fall in the gamma globulin occurred with progression of the disease

The pathological changes in the liver observed by biopsy or autopsy in 9 of the patients were not found to be specific Characteristically during the early stages of illness biopsies showed great numbers of mononuclear cells primarily in the portal areas (Figure 3) Studies of these cells (carried out in collaboration with Dr Robert Good) showed that as many as 40 per cent of the total were plasma cells and the number of the latter correlated grossly with the gamma globulin level in the serum Specimens obtained later in the disease showed a considerable decrease in the cellular infiltration and most of the patients showed a postnecrotic type of cirrhosis with broad bands of fibrous tissue between small and very large regenerating nodules

None of the features described above can be considered specific for these patients Individuals with cirrhosis after typical attacks of infectious hepatitis studied at the same time here and also by other observers [1, 5] show many of these manifestations The high gamma globulin levels and

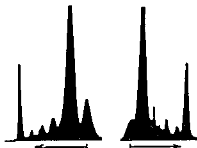


FIGURE 2 Tiselius electrophoretic pattern of the serum of the middle patient of Figure 1 The extreme elevation in the gamma globulin fraction is readily apparent

cellular infiltration of the liver certainly have been observed after hepatitis Perhaps they were somewhat more marked in the present group of patients with cirrhosis of undetermined etiology The high incidence of arthritis is somewhat peculiar for posthepatitis cirrhosis but again does not exclude this disease In essence therefore the only unique characteristic of this group of patients is the high incidence in females

Considerable effort was directed toward the determination of etiological factors other than infectious hepatitis The high incidence among young females and the generalized nature of the disease process in certain patients suggested the possibility of lupus erythematosus Positive L E preparations were consistently obtained in one patient but not in the others although it was not possible to test all of the patients particularly during the acute stage of their disease In view of the arthritis sheep cell agglutination tests were carried out in 5 patients with completely negative results However the recent modification of this test employing gamma globulin directly [3] was slightly positive in 3 of these individuals These are probably false positive reactions because of the high levels of many normally occurring antibodies along with the high gamma globulin No direct relationship between these cases and the above diseases could be established although these studies remain incomplete

In a previous report [2] preliminary evidence was presented for an effect of estrogen administration in raising the bilirubin level in the serum of some of these patients This observation has been confirmed and 4 of 5 patients tested showed an increase following 3 mg per day of diethylstilbestrol In some cases the estrogen made the patients very sick and they appeared to be peculiarly sensitive to the action of this drug In one case cortisone was taken by a patient for several years with symptomatic benefit and a fall in the bilirubin level On cessation of cortisone a prompt rise in bilirubin always developed Estrogen produced its



FIGURE 3 Photograph of a section of liver taken at laparotomy biopsy from one patient during the early stages of the disease Marked mononuclear cell accumulation is observed

effect in the presence of cortisone and caused nausea and vomiting on each occasion

These effects of estrogens which have not been observed in a limited control study of patients with other types of cirrhosis raise the possibility that specific endocrine influences present in young women modify the usual benign course of infectious hepatitis There is perhaps a relationship to the high incidence of fatal liver disease among postmenopausal women in Denmark [4] where the evidence for a type of infectious hepatitis is more definite However it is not possible at present to arrive at a conclusive

decision regarding the group of patients described here Single or multiple unrecognized etiological factors may well be involved

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DESIGNATED DISCUSSION

EDWARD A GALL, M D (Cincinnati Ohio) I have been delighted with this oasis of pathology The water is fresh and clear

Dr Hill has opened Pandora s box in broadening the horizon of hepatitis He permitted us to peek rather briefly at yellow fever and listed a number of other forms of infectious disease of the liver We have a growing acquaintance with a number of other related conditions An example in point is the hepatic lesion of infectious mononucleosis which so closely resembles that of the ordinary epidemic hepatitis I believe that the evidence indicates that mononucleosis is accompanied by hepatitis more often than not

In recent years as familiarity with the histologic pattern of hepatitis has increased I have been impressed with its occurrence in autopsy material procured from patients in whom no suspicion of liver disease has been entertained during life Notably this has been the case in association with certain forms of infectious encephalomyelitis and in children with *interstitial pneumonitis Hepatitis probably accompanies quite a number* of systemic viral infections not commonly considered to affect the liver

I presume from Dr Hill s illustrations (he did not clearly indicate this in his discourse) that the sclerosing venous disorder he has described has a centrilobular distribution I am frankly puzzled at the absence of a *residuum of an inflammatory reaction in association with the desmoplastic* process Indeed it seems rather odd that we do not see its equivalent following some cases of severe viral hepatitis since these very frequently exhibit inflammatory and even necrotizing change in the area of the central vein

I cannot quite ascribe to Dr Smetana s claim that the clusters or colonies of Kupffer cells constitute a characteristic of viral hepatitis These are not in my experience limited to this condition One sees them rather frequently in instances of severe extrahepatic biliary obstruction when they are particularly highlighted by bile staining I don t know about *their lipofuscin content here*

The observation of the acinar arrangement of parenchymal cells in the Delhi cases is interesting A similar although not as striking situation occurs in cases of hepatitis observed in this country Nor am I convinced that this is necessarily a specific indication of viral infection I am sure you are familiar with the emphasis placed upon acinus formation of hepatic cells in children suffering from galactosemia This is similar in many respects to what Dr Smetana has illustrated and has been claimed by some to be characteristic of galactosemia I do not believe it is pathognomic of any particular disorder

Dr Kunkel s reference to correlation of hepatic disorder with function

tests brings to mind a survey we have carried out recently We felt that there might be some parallel between the plasma globulin and particularly the gamma globulin fraction and the activity of the disease in the liver It seemed that the globulin might in some way indicate the number of reactive lymphocytes and plasma cells present in the liver We were unable to establish such a correlation There is probably sufficient extra hepatic hyperplasia of lymphoid tissues to maintain a high globulin level after the liver lesion becomes inactive

GENERAL DISCUSSION

Sheila Sherlock M D (London England) When I knew my friend Dr Kunkel was presenting a paper I thought it would be rather fun to collect the patients with cirrhosis that I see in England under the age of 1

I found I had seen patients with juvenile cirrhosis of which 13 fell into the group that Dr Kunkel has been describing and which we in England call Kunkel girls Actually that was not very well received by Dr Waldenstrom when he arrived from Sweden a he thought they ought to be Waldenstrom girls

Of the 13 Kunkel girls 6 were relatively well—exhibiting acne jaundice globulins plus and so on Six gave a past history of hepatitis and 7 did not The mean age was 16 and only 1 was under 13 There was this same sex incidence 1 females to 1 male

This certainly was not the only sort of cirrhosis that I saw in young people There were 9 others and the history of hepatitis in them was of about the same incidence as the other ladies—4 yes and 5 no—but the mean age which was 9 was much younger One was 1

This looked to me as if the Kunkel girls were suffering from much the same disease at a special age and anyone who contracted a posthepatic cirrhosis at the age of puberty would probably show this picture

The other thing to notice is that 7 of the others were in good health with no liver failure That is in contrast to the observation of Gelles that cirrhosis in children is a serious condition That has not been my experience Seven of these people had just hepatosplenomegaly or indica tions of portal hypertension and did reasonably well Two of them died however of liver failure

The rosettes of liver cells produce a pseudoductile appearance often with an inflammatory reaction between in fact Professor Diver felt this reaction to be much like his cases of neonatal hepatitis This can be found in patients with a good past history of infectious hepatitis So I think we will agree with Dr Gall that this may well be a nonspecific response of the liver

M Pjorner MD (Copenhagen Denmark) In connection with Dr

Hill s paper I would like to demonstrate the incidence of hepatitis in Denmark during a period of twenty eight years

Since 19 8 this disease has been notifiable in Denmark The registration is naturally associated with certain errors Not all cases are notified This was checked in 1946 by Reushing who found that 84 per cent of all cases of hepatitis diagnosed by doctors in Copenhagen during a period of six months were notified There is however no reason to suspect that this inaccuracy varies from year to year Incorrect diagnosis may also occur silent gallstones or cancer but the error is probably small

There were two big waves of hepatitis in the 30s and '40s covering several years The cause of this high incidence is unknown It might be that we had introduced a virus with a high virulence I shall point out that the increase in the epidemic in the 40s started with the invasion of the German troops in Denmark

The characteristic variation is pronounced especially in the years when there was a high incidence of hepatitis

LEO KRAINER, M D (Washington D C) During this morning s presentation Dr Schiff mentioned cholangiole In comparison with the number of instances of cases with a diagnosis of cholangiolitic hepatitis, his figures were rather small

I would like to make one point As regards the term cholangio historically it means two entirely different things In Rester s discussion on liver disease he speaks of the disease of the ultimate radicals and says In this case one likes to speak of cholangiolitis in terms of inflammation of the so called bile capillaries

Cholangioles and bile capillaries are identical and he stresses that it is very difficult to distinguish between the volume of bile capillaries and liver cells In other words bile capillary damage to him is liver cell damage

It has been used also by Eppinger who states that according to Naunyn the inflammation of the smallest ducts in the periphery is cholangitis He does not say expressly that he thinks so

This point of difference is of some practical importance in understanding the presence of capillary bile thrombi in various forms of liver disease and especially in the so called cholestatic hepatitis If we want to ascribe the presence of this bile thrombus to the portal spaces and alterations therein we use the older form of cholangiolitis but I think this static phenomenon cannot always be explained by the amount of damage in the periphery and I point to the fact that behind the term of cholangiolitis and cholangiolitic hepatitis there is something like liver cell damage

Dr Call pointed to the presence of lipofuscin in Kupffer cells in con

ditions other than viral hepatitis We have studied this with fluorescence and we are quite convinced that in cases of obstructive bile stasis there appears together with the bile a substance which resists digestion as shown with periodic acid Schiff (PAS) staining and which fluoresces in the same way as lipofuscin This indicates also in our opinion that in cases of obstructive bile stasis the bile stasis is not just a mechanical effect of obstruction but there is also a certain amount of liver cell damage

A M Rappaport MD (Toronto Canada) I must say I enjoyed Dr Baggenstoss s paper very much and he presented beautiful material which fitted so well with the acinar concept

I would like to make one point The position of the veins is usually described as outside the liver lobule and this is considered a patho logical feature From the material that we have shown we know that the so called central veins or portal hepatic veins are always between and at the periphery of the acini The nodules which are the result of hyper trophic regeneration of the surviving acinar cord are surely distorting these central veins compressing them and thus adding to the other cause which is the reduction of the number of peripheral vessels in this way portal hypertension is formed

I would like to make another suggested explanation for the increase of lymph vessels which Dr Baggenstoss described As I remarked in my paper we can imagine the liver from an embryological viewpoint as a giant web of parenchyma spread out on one side subdivided by the afferent portal vessels while on the efferent side the hepatic base inter digitates with them The moment a pathological process disturbs the communication between these two systems then the other efferent system that I mentioned and which is on the afferent side of this giant web the lymphatics and the bile ducts will probably be forced to carry away a part of the plasma fluid which is stagnating in the liver when normal passage from portal vein to hepatic vein is disturbed

First we will see an increase in lymph vessels which still will be able to carry on and do the job but when the amount of fluid to be transported is overtaxing this increased lymphatic net then transudation will occur the same way as it is produced by putting a constricting ring around the efferent lymph vessels surrounding the inferior vena cava

One question that I would like to ask Dr Hill is in his cases if he regularly observes a strict concentric fibrous ring around the terminal hepatic veins or whether sometimes he observes two stellate processes projecting into the hepatic parenchyma as I believe I observed in one corner on the slides he presented

Kenneth R Hill MD (Jamaica BWI) The answer to Dr Rappa

port s question is yes In veno occlusive disease the terminal advent is a nonportal cirrhosis The cirrhosis commences in the center of the lobule and goes out to the stellar process

One is Dr Smetana s collection of Kupffer cells with pigment This also happens in the recovery stage of yellow fever and they are called yellow bodies I don t know what the pigment is but it is presumed it is bilirubin or one of the bile pigments and it is possibly just a normal scavenging effect in the liver of the mesenchymal system

I have been most intrigued by the papers this morning which have a pathological flavor as it were The question is What is the virus doing in the liver When we look at yellow fever we know that the virus is in the parenchymal cells The virus itself has been isolated just recently in Trinidad from the liver cells This virus either kills the cells and also kills the patient or the patient recovers and as far as we know there are no aftereffects So it is a parenchymatous inflammation if you like

What happens in infectious hepatitis It is presumed (and others will tell us more about this) that the virus is in the cells What happens Dr Baggenstoss actually has rather shaken me in my concepts of what happens in posthepatitic cirrhosis I thought it was due to the strangling effect of the mesenchymal fibrous tissue afterwards but he suggests actually it is due to the hyperplasia of the liver cells which affect the blood stream

What of the hyperplasia? As a pathologist when I look at dead tissue in biopsies it never occurs to me Is the virus present? What is causing the hyperplasia? Is it a compensatory hyperplasia or is it virus present? I think it is a feature of research which could be well looked into that is the question of the virus present in the cell Is it or is it not strangling mesenchymal reaction?

HANS F SHITANA M D (Delhi India) I think the discussions have shown that we by no means understand each other very clearly There still seems to be a great divergence of opinion Perhaps it centers mainly on nomenclature There is considerable difference of opinion about nomenclature I believe that should be the first thing to come to an agreement on

This is not only an academic point but the diagnosis of viral hepatitis is still very shaky I have come across that in a very important disease of the East namely infantile cirrhosis The diagnosis of hepatitis in such cases is done with great frivolity without much evidence of hepatitis I am not saying that it does not exist but it has not been proven The big question is Is this disease due to virus or is it due to malnutrition? There is a great gap in our agreement concerning viral hepatitis and it remains for future studies to clear this point

Therefore the emphasis remains on certain criteria of hepatitis which

I believe still are furnished by histologic examination more so than by any other test

There are many other things which are important in this morning s discussion I would like to defend myself against the onslaught of Dr Gall about the lipofuscin in the Kupffer cells I meant lipofuscin not bile I believe that is important Also as I said lipofuscin does not occur in Kupffer cells in other conditions such as carbon tetrachloride poisoning In such cases of poisoning the other features of hepatitis are missing This presence of lipofuscin in Kupffer cells is only a part of the picture but it has to be lipofuscin and not bile pigment

Concerning the glandlike formations it is not the glandlike formation which also occurs as a malformation in the young child but it is the combination or the histologic feature of hepatitis and the glandlike formation which is the usual feature I believe that is specific because the features of hepatitis are present

Concerning the cholangioles that again is a very ticklish question It depends upon what city and country you are in Sometimes the very name cholangiole sort of makes the skin produce goose pimples One wonders whether it is necessary to deviate from the old term of pericanaliculus which is a very good name and is identical with cholangiole

As far as cholangiolitis is concerned this is supposed to be an inflammatory process of the cholangioles but the inflammatory process is never seen it is apparently just there The inflammatory expression is not obvious Therefore I don t know what it actually means

The presence of lymphatics is certainly very interesting I want to ask Dr Baggenstoss how he differentiates between the vascular and the lymphatic when there are two coats of muscle tissue This is rather important and I wonder how he is able to tell the difference with certainty

Dr Hill s picture of the occurrence of the veno vascular disease in India I think is somewhat erroneous because I have seen all his slides and I don t believe anybody agrees any more that this is vascular disease I think these cases were mistakenly diagnosed

A H Baggenstoss M D (Rochester Minnesota) I agree with Dr Smetana that the differentiation of lymphatics from venules is an important one particularly in view of the fact that in portal obstruction the accessory veins of Sappey are very well developed in the hepatoduodenal ligament.

The criteria we used mainly were those found in the textbooks in describing the lymphatics namely the muscular wall is poorly developed the muscular wall varies considerably in thickness in different portions of the wall the elastic lamina particularly the internal elastic lamina is

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PART II

Virology and Epidemiology

Moderator GILBERT DALLDORF M D (*Albany New York*)

very poorly developed and of course the presence of valves in the lymphatics which is of great help

In many of these cases the contents of the vessels also were of course of help in differentiating between the two

We are well acquainted with the accessory veins of Sappey because we studied the series of cases in which prolonged portal obstruction has been present and these are very well devolved in many of those instances

8

Etiology of Hepatitis

F O MacCALLUM M D
(London England)

This is the second time I have sampled the generosity of the Ford Hospital which has made it possible for me to attend such a stimulating discussion and to which I am extremely grateful

I was very doubtful about the propriety of my appearing on this program — particularly on this subject — when the suggestion was first made nearly a year ago I had nothing new to contribute and was particularly embarrassed at a later date to find Dr McLean following me on the program in view of the recently published work of him and his colleagues[1] However I shall speak about one or two aspects of hepatitis which may be covered by the heading Etiology

Many of us who were feeling somewhat jaded by 1950 as far as work on hepatitis was concerned were revived by the possibilities suggested by the work of Enders and his colleagues and the pure tissue culturists like Earle Gey and others but gradually became despondent once more when cultures of one tissue after another showed no response Of course we all hoped the answer was just around the corner

At a small conference held in March 1954 the up to date position of attempted transmission of virus hepatitis to laboratory animals and tissue culture of a number of lines of cells was discussed and the proceedings later appeared in Publication 322 of the National Academy of Sciences Washington[2] The results of a number of hitherto unpublished experiments using various experimental approaches came to light Various types of virus hepatitis occurring in mice dogs and other animals were discussed and it was concluded that although they were important in their own right they did not throw any direct light on the diseases in man at present known as infectious and serum hepatitis Following this meeting in order to fill in one gap I carried out serum neutralization tests with gamma globulin from the convalescent serum of dogs infected with canine hepatitis and Rift Valley fever virus in mice but found no evidence of cross relationship

However the resemblance and possible relationship of equine infectious anemia to serum hepatitis was considered to be worth further study, although it was stated that the virus of equine infectious anemia (E I A)

We will now consider briefly the subject of infection immunity and resistance to the two viruses which I will here call A and B

Of those patients who have an attack of infectious hepatitis in childhood it has generally been concluded from epidemiological studies that about 3 per cent to 5 per cent may have a second attack of infectious hepatitis with jaundice as adults The high rate of immunity is presumably due to the presence of adequate antibody Thus the gamma globulin fraction prepared from pooled adult serum (which is itself not infective) contains antibodies to the virus and can be used to protect or attenuate infection with virus A There is little or no evidence to suggest that the virus is carried in the blood stream for long periods The site of growth of the virus is somewhere in the gastrointestinal tract or reticuloendothelial systems or both Persistent nonicteric illness with continued excretion of virus in the feces in very young children has been reported If the patient who has recovered from infection with virus A is later infected with virus B there is no indication that his susceptibility is different from those without such a history

Unfortunately the information on immunity to reinfection with virus B is confined to observation on a small number of volunteers Although none of these became jaundiced on reinoculation there was slight clinical and biochemical evidence of disturbance of liver function in some of them following reinoculation [4] Other experiments with volunteers showed that neither convalescent serum [5] nor the gamma globulin fraction of a pool of serum collected 6 months to years after infection with virus B [6] protected against virus B The possibility that antibody to virus B is concentrated in some other fraction than gamma globulin has not been ruled out (I was interested to read recently that gastoenteritis of young swine apparently is not prevented by injection of gamma globulin prepared from convalescent serum)

The experimental work of Stokes and his colleagues and others has shown that carriage of virus B in the blood stream may persist for at least 5 years Some of the carriers have not shown any signs of liver disease detectable by biochemical tests or microscopic examination in liver biopsy Even if one admits that these tests are crude the evidence suggests to me that the virus may multiply in some organ other than the liver and immunity is obtained by persistent infection of the susceptible cells — a relatively harmless infection of the host This would be compatible with the suggestion that the lower age groups are less susceptible than older ones

If new laboratory tools confirm the lack of antibody production although there is ample gamma globulin in serum hepatitis does it mean that the structure of virus B is so similar to some normal constituent of the blood that plasma cell or other antibody forming mechanisms do not

was inactivated by heating at 60° C. for 1 hour in contrast to survival of serum hepatitis under the same conditions It was suggested that further experiments be carried out on susceptibility of horses not carrying E.I.A. virus to human serum hepatitis I have not heard if this has been done

It was agreed that the susceptibility of primates had not been investigated on a sufficiently large scale to conclude that they were completely insusceptible and it was suggested that further work using more animals per experiment should be done I believe that most virus laboratories have been so involved in tissue culture studies of viruses of all kinds including hepatitis that no one has had time for this We have tried to infect a small number of rhesus monkeys with a strain of *Shigella flexneri* isolated from monkeys plus hepatitis material with no positive results except some information on excretion of shigellae I have heard a rumor that a similar type of experiment has been performed in the United States using another enteric organism but I know nothing of the results

We like many other laboratories have been attempting to produce visible changes in tissue cultures of many kinds human embryo and monkey tissues in particular The latter included tissues from the monkeys mentioned above sacrificed at intervals afterward to compare their growth with that of tissues from normals All this has been unsuccessful and we are now testing a line of Detroit 6 cells obtained from Dr McLean

So far we have used the following materials in these Detroit 6 cells with negative results

1 Stools (a) from one adult collected on the 8th day of disease first day of jaundice
(b) from one child aged 6 years — specimen collected 8 days after onset of nausea and days after appearance of icteric tinge in sclera
2 Serum 034 A pool of human serums causing serum hepatitis in 60 to 70 per cent of volunteers when last tested in 1945 It has been stored in the dried state at 4° C. or lower temperature since 1945

The use of human serum seemed to be a perpetual stumbling block because of the possibility of virus or antibody being present My colleague Dr Margaret Walker has now succeeded in adapting Detroit 6 cells to grow without human serum The medium now in use is Synthetic Medium 199 of Parker with the addition of 10 per cent ox serum Cultures grown on this medium appear to be as satisfactory as those grown on the human serum recommended by McLean[3] but the latter states the cells will no longer be susceptible to hepatitis virus These negative results are obviously of little significance and Dr McLean and later speakers will no doubt enlighten you on the present position

In the past ten years there have been reports of a considerable number of cases of hepatitis in infants in the neonatal period born of mothers with no history of jaundice and in whom no other definite etiology such as congenital atresia of the bile ducts or Rh incompatibility was found. Groups of cases have been reported by Bodian and Newns,[13] Craig and Landing,[14] Dible *et al*,[15] Gellis and his colleagues,[16] Stokes, Wolman and their colleagues[17] and others. Unfortunately it is impossible to determine whether there is any difference in sex incidence from the incomplete information in most accounts. The cardinal features of these cases have been the appearance of jaundice at some time between birth and the twelfth week of life in the majority, some have been born several weeks prematurely with jaundice, a fatality rate of 30 to 40 per cent, and several examples of 2 or more cases in families. On microscopic examination of the liver large numbers of multinucleated giant cells and very active islands of hematopoiesis similar to those seen in erythroblastosis foetalis have been found, features not seen in the liver in virus hepatitis in adolescents or adults. The pathological material studied has been post mortem specimens from fatal cases or biopsy specimens usually obtained at laparotomy when, as is often the case, congenital obstruction of the bile ducts has been suspected. There has been no proof that the microscopic picture described was pathognomonic of a single etiology; in fact the multinucleated giant cells have been seen in other diseases affecting the liver in infants and the islands of hematopoiesis in erythroblastosis foetalis. This does not rule out the possibility that some of the infants had two diseases. Three of 20 cases whose livers (biopsy or post mortem) were considered by Craig and Landing to have the specific microscopic picture also had clinical and serological criteria of erythroblastosis due to Rh incompatibility, and in one pair of probable cases from one mother mentioned by Dible *et al* the multinucleated cells and fine fibrotic lesions were present but there were no signs of hematopoiesis. In another possible case mentioned by Dible and his colleagues cytomegalic inclusions were present in the kidney and pancreas.

In the discussion of Wolman's work in 1951,[17] Wolman and Zuelzer both mentioned that they had also seen specimens of liver from infants with suspected virus hepatitis in which the microscopic changes were similar to those seen in adult virus hepatitis. No mention is made of multinucleated parenchymal cells in the case of fulminant hepatitis in an 8 week old infant described by Williams and Gaber,[18] the four siblings described by Adams *et al*,[1] the case whose mother probably had infectious hepatitis described by Toscano and Rossi in Italy,[12] nor in four cases described by Perry in Australia.

I have been unable to find any other more recent detailed reference to this latter point, which seems important. As several authors have asked

consider it a foreign substance and are not stimulated to antibody production. Or is it because infection first occurred *in utero* that no antibody response occurs in some of the individuals? Were the volunteers and others who did not show illness when injected with large doses of virus B already carrying the virus? If so, their number suggests a much higher carrier rate than 1 in [illegible]00 in England and Wales—*a figure deduced from* the hepatitis rate following whole blood transfusion.

In contrast to the outcome when virus B follows virus A, observations made during the 1939–1945 war in the Mediterranean region suggested *that previous infection with virus B* rendered an individual more liable to clinical disease with strains of virus A present in that area than were normal individuals.[7] It is possible that persisting virus B sensitizes liver cells by primary infection, interferes with the capacity of the liver to deal with virus A, or interferes with antibody production.

Finally, in support of the idea that there are hepatitis viruses with different properties, I have found it difficult to discard the evidence provided by *three different workers, MacLagan,[8] Neefe[9] and Green,[10] that* the thymol and colloidal gold flocculation tests of liver function gave different results in infectious hepatitis and serum hepatitis.

The second subject is Fetal and Neonatal Hepatitis and Hepatitis in Infancy.

I wish first to acknowledge the published reports of the Boston and Philadelphia groups who have done so much work in this field and no doubt have more recent data which I have not seen.

If we consider there are two types of virus, both of which may circulate in the blood stream (and one at least—that of serum hepatitis—having a diameter of less than 50 millimicron), one may conclude that it is possible for the fetus to be infected if the mother is. This presumably could result in death *in utero* from either virus. I have found no information on results of examination of such fetuses. If the mother has infectious hepatitis and infects the fetus, I suppose the baby would be more likely to be born alive and jaundiced if this infection occurred in the last month of pregnancy, though not necessarily so. There are very few published reports of probable *in utero* infection with infectious hepatitis, of which those of Olin[11] and Toscano and Rossi[12] are examples. No doubt Dr Martini of Hamburg may have something to say on the subject as he, as well as others, has followed up a number of cases of jaundice in pregnant women, all with negative results.

On the other hand, I suppose that if the mother is already a carrier of *serum hepatitis* virus, or becomes infected during pregnancy with it, and the fetus is infected, the infant might first show symptoms and signs any time from birth up to 3 or 4 months. It has been suggested that such a child might become a carrier and, if a female, the condition might be perpetuated in this way.

Finally on a more clinical topic one fact that still amazes me is that physicians and surgeons throughout the world continue to inject untreated pools of serum or plasma from a large number of donors for some quite unnecessary procedures in addition to unnecessary transfusions As recently as July 1956 at the International Pediatric Congress I listened to a paper on the use of human serum as antigen for skin tests in rheumatic fever The speaker said he was aware of the risk of serum hepatitis and had tested his material on a few volunteers first, but I think this type of work should be discouraged until a laboratory test is available

There certainly are a very large number of interesting and practical problems awaiting solution by the laboratory workers

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Is there yet another virus disease of the liver in infants or even perhaps several more

There is little information on the possibility of the occurrence of mild cases which may have occurred at home and recovered except when there have been inquiries as a result of fatal cases in siblings

Gellis and his colleagues obtained no information to suggest hepatitis in 5 siblings who were born before the primary case and in only 1 of 7 who were born after the primary case Although Stokes *et al* Gellis *et al*, and Craig and Landing appear to have seen very few multiple cases in families a number of examples have been described particularly in England [13, 15] The reason for this difference is not obvious

I understand that the erythroblastosis foetalis rate is somewhere in the neighborhood of 1 in 200 pregnancies in England and Wales It is odd that this should be the same figure as one suggested for virus B carriers! *I have not found any report from a large maternity and pediatric unit of* cases of jaundice divided into those with and without erythroblastosis I hope that information on this is available from some large units in the United States and that such information will be forthcoming in Great Britain If one accepts the idea that at least 0 5 per cent of donors are carriers of virus B it would be interesting to know how many mothers actually do produce infants with hepatitis correlate this with the calculated carrier rate for the particular country and study other possible factors associated with clinical disease in the infant To conclude my remarks on this aspect I would like to mention a situation which arose about two years ago I had made an attempt to obtain the interest of two maternity units in one of which the pathologist was interested in kernicterus in order to collect serums from mothers and infants when the infants had jaundice in the neonatal period not due to erythroblastosis or other known causes I also asked if they could do liver function tests on all mothers The serums were to be tested for herpes simplex antibody and held for virus hepatitis studies The net result was one case the third child of a family in which two previous infants were said to have died with jaundice in the neonatal period I happened to be carrying out follow up with a request for a third serum at the end of a year because there had been herpes antibody in the first at the time when the invitation to this symposium arrived I inquired about the outcome of the child of whom I had not heard since the first month of life and also if I might mention this interesting sequence of three presumed virus hepatitis cases The answer was that this child had been a case of galactosemia As soon as the sugar was removed from its diet it quickly recovered Perry [20] mentioned a similar incident in Melbourne where the first infant died of presumed virus hepatitis but the second was recognized as having galactosemia was treated and recovered

9

Tissue Culture in the Isolation of the Hepatitis Virus

I WILLIAM McLEAN JR MD
Detroit Michigan

The problems which have been surmounted in determining the etiology of infectious and serum hepatitis have just been reviewed by Dr F O MacCallum On the basis of human volunteer studies nearly everyone agrees that the evidence is quite convincing that the causative agents should be classified as viruses To date however little progress has been made in their laboratory cultivation in spite of the exacting and thorough work by a number of competent laboratory groups For almost two years our laboratories have been engaged in study of this problem and for your evaluation some aspects of this work will be reviewed at this time Much of the data cannot as yet be fitted into a neat pattern of facts and more question will be raised than answered but the findings related to the etiology of hepatitis are certainly suggestive and worthy of further study in other laboratories

Recently a preliminary note has been published[3] describing the early findings with a group of transmissible cytopathogenic agents derived from serum and fecal specimens of cases diagnosed as acute infectious hepatitis The first of these agents (MR_1) produced degenerative changes on a mixed culture of cells from human testicle (PD39T) that had been isolated in our laboratory It is important to note at this point that this cell line was found to be quite unstable and could not be maintained Later we were able to reisolate from the same serum and pass the MR_1 agent on an epithelial cell line (Detroit 6) derived from human bone marrow[1 4]

The first isolate has now been maintained on Detroit 6 for fourteen serial passages and has retained its original cultural characteristics In addition to MR_1 the original group of isolates previously described[3] has been passed and additional isolations have been made bringing the present established total to 43 All of these agents show a similar and characteristic cytopathogenic effect (CPE) In spite of the fact that materials for study have come from widely divergent geographical locations (Germany Canada England India and different sections of the United States) and

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to be somewhat of a pattern in the isolations but the negative results during the early clinical phase of the disease were not anticipated However the series is still small and any speculation at this time would be premature

The laboratory procedures currently being employed will be published in detail elsewhere but a few comments are in order The growth medium for the cells consists of 40 per cent human serum and per cent chick embryo extract in a balanced salt solution For tests or passages the cultures are washed and changed to 10 per cent horse serum in medium 199 In this maintenance medium after about ten days control cultures will usually show granularity some rounding and disruption of the normal sheet configuration These changes due to accumulation of metabolites and inadequacies of the medium unless anticipated are easily confused with the specific cytopathogenicity which they resemble superficially Figure 1 illustrates the characteristic change in appearance of positive cultures The control on the left is typical of a healthy cell sheet On the right distortion and disruption of the cell sheet are beginning The individual cells are contracting rounding up and beginning to aggregate Very little change is noteworthy at the cellular level and inclusion bodies have not been demonstrated

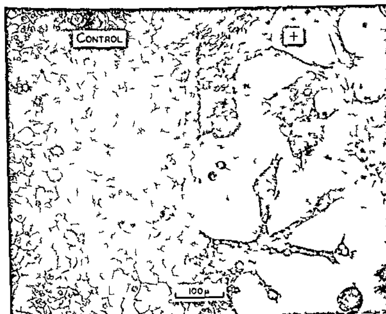


FIGURE 1

have been collected over a span of about ten years the isolations have all been remarkedly similar in their cultural characteristics and other properties It should be emphasized that this group of 43 isolates does not include reisolations from the same specimens and only those isolations in which the characteristic CPE can be transmitted serially are considered valid

The isolation rates we have obtained with acute and convalescent specimens from cases of infectious hepatitis (IH) and with samples from serum hepatitis (SH) are shown in Table 1 The finding of 13 positives in tests on 16 acute serums and 10 of 13 for stool extracts has been encouraging Using the same procedure only 9 of 33 convalescent serums have been found to contain agents but these isolates appear similar in every way to those derived from acute phase specimens These ratios give a corrected X value of 14 5 indicating that it is extremely improbable that the difference in isolation rate could be due to chance This value of approximately 5 per cent apparent isolations from convalescents is noteworthy as it corresponds closely with the apparent carrier rate in the general population Later in connection with tests on a much larger series of so called normal serums the importance of this finding will be discussed

Our experience in testing specimens associated with serum hepatitis has been less extensive Samples of 3 materials derived from human blood and definitely known to have caused typical serum hepatitis when administered to patients were all positive in our tests The remaining specimens with one exception were supplied under code by Dr Roderick Murray of the National Institutes of Health The serums were from 4 representative cases of serum hepatitis and were taken at different periods following known exposure by inoculation Samples from the icteric phase were negative while specimens obtained during the incubation period were positive as were those taken in early convalescence Four serums from later convalescence were again negative Obviously there appears

TABLE 1

ISOLATIONS FROM HEPATITIS-ASSOCIATED SPECIMENS

	Type Source	*Total Tested*	*Isolation of Agent*
Infectious Hepatitis	Acute serum	16	13
	Acute stool	13	10
	Convalescent serum (1–12 mos)	33	9
Serum Hepatitis	Products causing	3	3
	Incubation period	5	4 and 1?
	Icteric phase	5	0
	Early convalescence	3	3
	Convalescence (3–4 mos)	4	0

serial passages of the agents since it indicates the necessity of mechanically disrupting the cells by freezing and thawing or grinding if the CPE is to be maintained on passage of cell free supernates or filtrates

In addition to the tests on coded SH samples we have tested similar IH materials submitted by other groups Table 2 shows results of tests on samples from Dr R McCollum of Yale University The first two samples were unpassed seed we had previously sent to Yale and samples 7 8 and 1 were Yale passages of our agents Y3 and Y10 are first and second passages respectively of a presumptive isolation made by Dr McCollum from an IH stool specimen It is of interest that we observed atypical degenerative changes in the Y3 sample which was possibly due to some residual toxic substances from the fecal material The second passage gave typical results Four samples of Detroit 6 control fluid (Y4 6 9 and 11) were submitted Our results are in close agreement with Dr McCollum's except for Y9 which was positive in Detroit but had been negative at Yale Convalescent serum passage (Y5) and a control of horse serum medium (Y13) were clearly negative

Similarly a study on samples submitted by Dr F Deinhardt from the Children's Hospital in Philadelphia is given in Table 3 Samples 3 4 5 and 9 were from the second tissue culture passage in Philadelphia of cultures originally seeded at Parke Davis with stool specimens from cases of acute infectious hepatitis Duplicate cultures retained in Detroit had resulted in isolations from To 7 and Pj but the other two fecal specimens had not yielded transmissible CPE Samples 1 and 8 were passages of two of our isolates and 2 6 7 and 11 were from uninoculated

TABLE

TESTING OF CODED LABORATORY SAMPLES RECEIVED FROM DR R McCOLLUM YALE UNIVERSITY

Sample Number	*Decoded Description of Sample*	*Results (Day Off)*
Y1	New A TC_4, PD	++++ (2)
Y2	MR 1 TC_8, PD	++++ ( )
Y3	IH stool Yale TC_1	++++ (7) *
Y4	Detroit 6 control Yale positive	++++ (7)
Y5	IH convalescent serum Yale TC_1	0 (7)
Y6	Detroit 6 control Yale negative	0 (7)
Y7	New A TC_4 Yale TC_2	++ ( )
Y8	MR 1 TC_8, Yale TC_2	+++ ( )
Y9	Detroit 6 control Yale o	++–+++ (7)
Y10	IH stool Yale TC	+++ (6)
Y11	Detroit 6 control Yale ?	+ ( )
Y12	Pas TC_6, Yale TC_2	+++ (7)
Y13	10% horse serum + #199	0 (7)
—	Cell control	0 (7)

*Sample moderately toxic [illegible] first passage

Similar changes at a lower magnification are shown in Figure 2 The degree of degeneration illustrated in the center panel is graded as one to two plus depending on the over all appearance of the culture On the right is a later stage in the degenerative change and it would be classified as 3 plus to 4 plus by the degree of spontaneous sloughing of the cells from the glass surface Note the cells are rounded and markedly clumped This clumping usually progresses and the aggregated masses are easily displaced so that in a strongly positive test the agglutinated cells can be observed readily by gross inspection of the gently agitated racks of tubes There is very little disruption of cells and even in this condition they continue to metabolize as evidenced by production of acid metabolites If such cultures are maintained these cell aggregates resettle on the glass and from each clump new outgrowths of cells appear If incubation is continued a new sheet of cells will form Also if a media change is made before marked clumping has occurred the aggregation will apparently be reversed and the cell sheet revert to a more normal appearance The fact that the cells clump rather than disrupt is also important in making

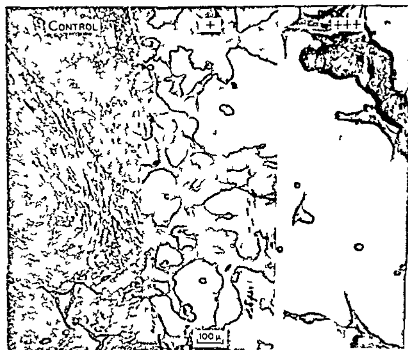


FIGURE 2

serial passages of the agents since it indicates the necessity of mechanically disrupting the cells by freezing and thawing or grinding if the CPE is to be maintained on passage of cell free supernates or filtrates

In addition to the tests on coded SH samples we have tested similar IH materials submitted by other groups Table 2 shows results of tests on samples from Dr R McCollum of Yale University The first two samples were unpassed seed we had previously sent to Yale and samples 7 8 and 1 were Yale passages of our agents Y3 and Y10 are first and second passages respectively of a presumptive isolation made by Dr McCollum from an IH stool specimen It is of interest that we observed atypical degenerative changes in the Y3 sample which was possibly due to some residual toxic substances from the fecal material The second passage gave typical results Four samples of Detroit 6 control fluid (Y4 6 9 and 11) were submitted Our results are in close agreement with Dr McCollum s except for Y9 which was positive in Detroit but had been negative at Yale Convalescent serum passage (Y5) and a control of horse serum medium (Y13) were clearly negative

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TABLE 2

TESTING OF CODED LABORATORY SAMPLES RECEIVED FROM DR R McCOLLUM YALE UNIVERSITY

Sample Number	*Decoded Description of Sample*	*Results (Day Off)*
Y1	New A TC_4 PD	++++ (7)
Y2	MR 1 TC_8 PD	++++ ( )
Y3	IH stool Yale TC	++++ ( )
Y4	Detroit 6 control Yale positive	++++ ( )
Y5	IH convalescent serum Yale TC_1	0 (7)
Y6	Detroit-6 control Yale negative	0 ( )
Y7	New A TC_4 Yale TC_2	++ (7)
Y8	MR 1 TC_8 Yale TC	+++ ( )
Y9	Detroit-6 control Yale o	++–+++ (7)
Y10	IH stool Yale TC_2	+++ (6)
Y11	Detroit-6 control Yale ?	+ (7)
Y12	Pas TC_8 Yale TC	+++ (7)
Y13	10% horse serum + [illegible]	0 ( )
—	Cell control	0 (7)

*Sample moderately toxic on first passage

TABLE 3

TESTING OF CODED LABORATORY SAMPLES RECEIVED FROM DR F DEINHARDT CHILDREN'S HOSPITAL, PHILADELPHIA

Sample Number	*Decoded Description of Sample*	*Results (Day Off)*
PA 1	Pas TC_{12} Phila TC_1	+++ (4)
PA 2	Detroit 6 control Phila TC_2	+++ (4)
PA 3	To-7 positive PD Phila TC_2	+++ (5)
PA 4	To-12 negative PD Phila TC_2	+++ (6)
PA 5	Pj 2 positive PD Phila TC_2	++ (6)
PA 6	Detroit 6 control Phila TC_2	+++ (5)
PA 7	Detroit 6 control Phila TC_2	++ (6)
PA 8	MR_1 TC_{10}, Phila TC_1	+ (6)
PA 9	Pj 7 negative PD Phila TC_2	+ (6)
PA 10	HeLa control	0 (8)
PA 11	Detroit 6 control Phila TC_2	0 (8)
PA 12	B cell control	?*
—	Cell control PD	0 (8)

* Odd cells overgrew Detroit-6 and replaced them in the culture

cultures of Detroit 6 cells handled in Philadelphia in the same way as the seeded cultures. The finding of typical degeneration in three of these control cultures probably explains at least in part the poor results obtained by the Philadelphia group. Similar control problems have occurred in our studies and will be discussed shortly. Passages 10 and 12 were control fluids from frozen and thawed cultures of malignant cells — 10 were from HeLa and 12 from a cell originating from human leukemic bone marrow. Both of these controls were clearly negative but it is evident that the B cells were not killed by the freezing and thawing since they grew out and replaced the Detroit 6 cells in our cultures.

From these two studies it appears that the problem of obtaining reproducible results in different laboratories has not been solved. It is evident that both at Yale and Philadelphia there have been problems in maintaining control cultures in good condition and this naturally has resulted in some discouragement. Spontaneous degenerative changes as well as apparent loss of sensitivity of the cultures have been the difficulties in establishing the system in other laboratories. We have been able to partially control these factors in our laboratory as will be discussed but they cannot be minimized as a serious drawback to the extensive application of the technique at this time.

Dr Joseph L. Melnick of Yale University has supplied under code samples of acute and convalescent serums from an outbreak of epidemic infectious hepatitis (Table 4). Agents were obtained quite readily from 6 of the 8 acute serums and less definitely 5 of the 15 convalescent specimens (19 to 40 days) were positive. The extent of CPE appears to

TABLE 4

TESTING OF CODED ACUTE AND CONVALESCENT IH SERUMS RECEIVED FROM DR J MELNICK YALE UNIVERSITY

Sample	*Decoded Description* *	*Results* †
3719	Patient 1 Acute	+++
3909	Patient 1 Convalescent	0
3720	Patient 2 Acute	+++
3910	Patient 2 Convalescent	+?
3735	Patient 3 Acute	0
3915	Patient 3 Convalescent	0
3733	Patient 4 Acute	0
3917	Patient 4 Convalescent	0
3622	Patient 5 Acute	++++
3935	Patient 5 Convalescent	++
3596	Patient 6 Acute	+++
3938	Patient 6 Convalescent	0
3586	Patient 7 Acute	+++
3945	Patient 7 Convalescent	0
3617	Patient 8 Acute	+++
3946	Patient 8 Convalescent	0
3952	Patient 9 Convalescent	++
3954	Patient 10 Convalescent	0
3963	Patient 11 Convalescent	0
3969	Patient 12 Convalescent	0
3981	Patient 13 Convalescent	+
3982	Patient 14 Convalescent	+
3983	Patient 15 Convalescent	0? (TC_1 +++)
Detroit 6 passage control and cell control		0

* Acute 3 to 8 days paired convalescent 30 to 40 days convalescent 19 to 40 days

† Preliminary result based on 2 passages for final report see Addendum

be more marked and the isolations more definite from the acute patients However these recorded results are based on only two passages They were decoded prior to the usually required three passages (negative or positive) in order to have the data for presentation at this time Additional studies are in progress and it is anticipated that they will confirm and extend these preliminary results, but even so there is little doubt that this study alone strongly indicates the validity of the technique Of special interest is further work on sample P3983 which showed a marked

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Sample Number	*Decoded Description of Sample*	*Results (Day Off)*
PA 1	Pas TC_1 Phila TC_1	+++ (4)
PA 2	Detroit 6 control Phila TC_2	+++ (4)
PA 3	To- positive PD Phila TC_2	+++ (5)
PA 4	To-12 negative PD Phila TC_2	+++ (6)
PA 5	Pj 2 positive PD Phila TC_2	++ (6)
PA 6	Detroit 6 control Phila TC_2	+++ (5)
PA 7	Detroit 6 control Phila TC_2	++ (6)
PA 8	MR_1 TC_{10}, Phila TC_1	+ (6)
PA 9	Pj 7 negative PD Phila TC_2	+ (6)
PA 10	HeLa control	0 (8)
PA 11	Detroit 6 control Phila TC_2	0 (8)
PA 12	B cell control	?
—	Cell control PD	0 (8)

Odd cells overgrew Detroit-6 and replaced them in the culture

cultures of Detroit 6 cells handled in Philadelphia in the same way as the seeded cultures The finding of typical degeneration in three of these control cultures probably explains at least in part the poor results obtained by the Philadelphia group Similar control problems have occurred in our studies and will be discussed shortly Passages 10 and 11 were control fluids from frozen and thawed cultures of malignant cells — 10 were from HeLa and 11 from a cell originating from human leukemic bone marrow Both of these controls were clearly negative but it is evident that the B cells were not killed by the freezing and thawing since they grew out and replaced the Detroit 6 cells in our cultures

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an alternate cell line and Table 5 summarizes these trials. Cells from 11 different tissues of a number of human embryos have been tested but at the time these data were compiled only the cell culture PD39T had been found to be sensitive. HeLa, amnion and possibly Detroit 116 have shown some promise but the results have not been consistent or clear cut nor have we been able to maintain CPE on passage.

The best control has been obtained by pretesting each batch of serum before preparing the pools to be used for cell cultivation. Table 6 gives

TABLE 5

TESTS OF T.C. ESTABLISHED CELL LINES AND PRIMARY OUTGROWTHS FOR SENSITIVITY TO HEPATITIS AGENTS

Tissue and Source	*Number of Tests*	*Results* 0	?	+
Human embryo (Parke Davis)	21	18	2	1
Lung, skin, testicle, liver, kidney, spleen, trachea, uterus, adrenal, etc. (Parke Davis)				
HeLa (Syverton and Microbiol. Assoc.)	12	3	5	4
Human amnion * (Parke Davis)	18	11	5	2
Detroit 116 (Burman, Stulberg)	13	10	1	2
Detroit 96 (Burman, Stulberg)	13	12	0	1
KB (Eagle)	3	1	1	1
MAF (Microbiol. Assoc.)	4	2	1	1
Detroit 6 Calf (McCollum)	4	2	1	1
Human liver (Chang and Microbiol. Assoc.)	7	5	2	0
Detroit 56 (Burman, Stulberg)	7	7	0	0
Chang conjunctiva (Microbiol. Assoc.)	2	2	0	0
Intestine (Microbiol. Assoc.)	4	4	0	0
He II (Melnick, Moore)	2	2	0	0
Human liver (Parke Davis)	2	2	0	0
Monkey kidney (Parke Davis)	3	3	0	0
Chick embryo (Parke Davis)	1	1	0	0
Detroit 6 (Microbiol. Assoc.)	1	1	0	0

* Primary outgrowths

TABLE 6

SUITABILITY OF SERUMS SUPPLIED BY 5 BLOOD SERVICES FOR CULTIVATION OF DETROIT 6 CELLS

Blood Service	*Number of Units*	*Unsatisfactory* Positive	Toxic	*Satisfactory* Number	Per Cent
JP	114	24	3	87	76
DB	18	47	10	175	69
MR	193	29	5	159	82
SM	118	8	1	89	75
HB	123	3		93	6
Summary	30	151	6	553	76

and early CPE on first passage and then was negative on second passage This pattern is not uncommon for isolations from convalescent serum and frequently on further transfers a transmissible agent may be established producing a typical CPE. We believe this type of isolation pattern may be due to the presence of both high concentrations of antibody and agent in the same serum sample It can be duplicated by artificial antiserum-virus mixtures as will be shown in the neutralization test results (Note Addendum p 168)

Spontaneous degeneration which is on occasion associated with pick up of a transmissible agent and what seems to be spontaneous loss of sensitivity of the Detroit 6 cultures are major problems Unless they are recognized and controlled the validity of experimental work may not be accurately interpreted Both of these problems as previously mentioned have been seen by us and they have occurred in other laboratories trying to duplicate our work It seems not unlikely that they are both related to the requirement of high concentrations of human serum in the growth medium It is well known from the epidemiological data that there must be a high carrier rate of hepatitis inducing agents in serums from the normal population therefore this could be the basis of the problem It has been a practice in our laboratory to maintain multiple cell lines of important cultures on media from different sources and only because of this has a sensitive culture of Detroit 6 cells been retained Culture lines have been lost from spontaneous degeneration during passage possibly because of the chance presence of virus in the growth medium Other lines after variable periods of poor growth recover but with loss of sensitivity possibly in this case the more susceptible cells in the population have been destroyed by a chance pick up while the more resistant cells have repopulated the culture Lines growing well in one pool of human serum may spontaneously degenerate when transferred to horse serum maintenance medium or a new pool of human serum We explain this on the basis of the presence of both virus and antibody in the human serum pool used for growth — the cells being infected but the effect of the virus being masked until the antibody is removed by replacing the medium All of these naturally occurring phenomena have been duplicated experimentally by passing Detroit 6 in the presence of various combinations of positive and antibody containing serums

To eliminate these difficulties the obvious solution is to adapt Detroit 6 cells to artificial media or at least to media without human serum However to date all such adaptations tested have not been suitable for use because of reduced sensitivity Table 5 includes the results of tests on one such line adapted to calf serum medium by Dr McCollum This line is not sensitive the one test recorded as positive was not typical CPE and could not be passed or reproduced Another possibility has been to find

specimens If additional blind passages were made of both the positives and negatives the relationship might be considerably sharpened

Over 200 probable isolates are now available for study Included are those from hepatitis associated specimens normal serums spontaneously degenerated control cultures and isolates from other laboratories These isolates all produce characteristic degenerative changes in sensitive cultures of Detroit 6 cells but beyond this they have not been classified The properties of some of the isolates from hepatitis cases have been studied but much remains to be done After thorough homogenization to break up the cell debris the agents were passed easily through bacteria retaining filters but so far neither ultracentrifugation nor electron microscopy have contributed additional information on size Attempts by several routes to produce pathology in embryonated eggs newborn and adult mice and rabbits have all been unsuccessful In addition it has not been possible to produce antiserum in experimental animals The small quantity of antigen produced in the cultures — infectivity rarely exceeds 10^{-} — may well account for these failures Before continuing studies of this type better methods of cultivation must be developed

Some interesting work with heat inactivation is in progress The thermal inactivation point seems to be at about 75 C Inactivation at 60 C is very slow with trace amounts of activity sometimes demonstrable after 20 hours in some preparations though usually activity is lost in about eight hours A recent finding is an apparent tenfold to thirtyfold increase in activity after heating at 60 C for one or two hours Incidental to this observation we have retested one of the negative serum specimens from acute IH (listed as negative in Table 1) after heating at 60 C for one hour An apparent isolation has been made and is being passed Two previous isolation attempts with this serum without the preheating were negative through three blind passages This observation if confirmed on further study might be of considerable value in cultivating and testing for agents of this group

Neutralization of the characteristic CPF can be readily demonstrated in convalescent serums from IH but limited neutralization tests on convalescent SH serums so far have not yielded definitive data

Table 8 summarizes the protocol of a typical neutralization test with a 14 month convalescent serum from a case of IH supplied by Dr Joseph Boggs of the Children's Memorial Hospital Chicago Half log serum dilutions were tested against three typical isolates from acute IH specimens Cultures are read daily but only those on the fourth and seventh days are recorded The MR_1 test is fairly straightforward No CPF in the test or control had shown up on the fourth day but on the seventh day a clear cut neutralization endpoint at about 1 30 was evident The Pas agent was neutralized at 1 1000 on the fourth day but virus breakthrough

the results of tests on serum from 730 pints of blood obtained from five blood services in the Detroit-Chicago area The test used was to attempt to grow known sensitive Detroit 6 cells in a 40 per cent concentration of the test serum A total of 151 positives has been found Positive in this sense means the cells grew out and then spontaneously degenerated or degenerated when changed into horse serum maintenance medium An additional 6 are listed as toxic indicating that no cell growth occurred Passages of most of these possible isolates have not been attempted nor have tests on other cell lines or in animals been made to exclude known viruses such as the Echo group Also some of the toxics could very likely be reclassified as positives if passaged But in spite of all this it is interesting that the ratios of satisfactory serums are quite consistent from group to group and correspond closely to the percentage of negatives found by more elaborate procedures on the IH convalescent serums Since using only such pretested serum in the cell cultivation there have been fewer unsatisfactory tests and loss of cultures Currently in progress are control passages of frozen thawed and mechanically ground Detroit 6 cells that have remained negative after five serial passages in such a series of cultures isolations from clinical specimens have been made and maintained without difficulty However the problems are not completely eliminated by this procedure and multiple cell lines are still being maintained to insure continuation of the study

The data presented in Table 7 must be considered as preliminary because of the small numbers involved but the trends indicated are of considerable importance It appears that the ratio of CPE positives may be proportional to the bilirubin content of the serum Bilirubin *per se* does not affect the cells A similar relationship to thymol turbidity has been demonstrated but this so far is based on only one passage of the serum

TABLE 7

RELATION OF BILIRUBIN LEVELS AND THYMOL TURBIDITY OF NORMAL SERUMS TO PRODUCTION OF CPE ON DETROIT 6 CELLS

	Range of Serum Bilirubin mg %			
	<0 5	*0 5–1 0*	*1 0–1 5*	*>1 5*
Number tested	5	2	6	4
Positive *	0	1	3	3

	Thymol Turbidity	*Shank Hoagland Units*	
	2	*4–8*	*10+*
Number tested	28	12	7
Positive †	6 (21%)	4 (33%)	3 (43%)

* Confirmed by passage
† Not passed

TABLE 9

HOMOLOGOUS NEUTRALIZATION DEMONSTRATING PROZONE-LIKE EFFECT AT LOW SERUM DILUTIONS

Serum Dilution	*Pom 11 Months Convalescent Serum vs Pom TC_{10}*	*Pom 11 Months Convalescent Serum vs Gar TC_6*	*Car 10 Months Convalescent Serum vs Gar TC_6*
1 4	++++	++++	0000
1 8	++00	0000	0000
1 16	+000	0000	0000
1 32	0000	0000	0000
1 64	0000	0000	0000
1 128	0000	0000	—
V C	+++0	+++0	++++
SC (1 4)	0000	0000	0000

The antigens used were typical isolates from cases of infectious hepatitis The first serum (RK_1) was obtained prior to starting the work on hepatitis and the second (RK_2) from the same person during early convalescence from a febrile disease which developed 10 months after starting work with these agents No jaundice or evidence of hepatic involvement occurred and the diagnosis of infectious mononucleosis was made on the basis of the laboratory and clinical findings The remaining three serums were obtained at the same time as RK_1 Note the different patterns of neutralization RK_1 and HJ did not inhibit the CPE of any of the agents FT neutralized but the most striking result is the comparison of RK and PS This was our first indication of serological distinctions between agents of this group Incidentally RK now exhibits high level antibodies to both MR_1 and Pas

The type of neutralization test just illustrated has been used on a larger scale (Table 11) to demonstrate antigenic differences and similarities between a number of representative passaged agents and CPE producing serums or stool extract before tissue culture passage This technique has serious limitations but at the present time it is the only means available for indicating that there are distinct serological groupings of these agents A to F represent five serums tested at a 1 50 dilution against 16 virus samples representing a broad selection of isolates at various stages of tissue culture passage The serums are A 3 months convalescent SH B 9 months convalescent IH (Yale) C 5 weeks convalescent IH (Germany) D normal serum (Table 10) known to inhibit MR_1 and Pas E a later bleeding from RK (taken 10 months after RK) and F a normal serum previously testing negative vs MR_1 and Pas The first eight antigens appear to make up a rather homogeneous group All are neutralized at least partially by the known antiserums A through E The

TABLE 8

NEUTRALIZATION TEST IH CONVALESCENT SERUM HAR (14 MONTHS)

Agent and Passage (Fourth and Seventh Day Readings Reported)

Serum Dilution	*MR 1 TC_{12}* 4	7	*Pas TC_{13}* 4	7	*Gar TC_{11}* 4	7	*Controls Serum* 4	7
1:3.2	—	—	—	—	—	—	+++	—
1:10	000	000	000	+++	+++	+++	000	000
1:32	000	000	000	++0	+++	+++	000	000
1:100	000	000	000	++0	000	000	—	—
1:320	000	++0	000	+++	000	000	—	—
1:1000	000	++0	000	+++	—*	—	—	—
1:3200	000	+++	++0	+++	000	+++	—	—
1:10 000	000	+++	+++	+++	000	+++	—	—
Controls	000	+++	+++	+++	000	+++	000 Cell	000

* Cultures contaminated with yeast, not readable.

occurred in nearly all the tubes by the later reading. To get reproducible results we have found it necessary to read the tubes daily and determine neutralization when the virus controls are showing about one to two plus degeneration. The "Gar" test illustrates the curious prozone-like results that have been observed with certain virus-antiserum mixtures. The appearance of a strong "positive" in the higher serum concentrations in about two days with negative serum controls and before the virus controls show any change is characteristic. We cannot explain this phenomenon but it seems to be associated in some way with the formation of an antigen-antibody complex and it is only seen when the serum neutralization titers are quite high. Similar CPE responses have been noted in isolation attempts on convalescent serums and on passage usually an isolation can be confirmed. Please note that this convalescent serum showed an early CPE in the 1:3 dilution of the serum controls. Subsequent blind transfers of these controls resulted in a definite isolation after two questionable or plus-minus passages. Unfortunately, the supply of this particular antiserum has been exhausted so neutralization of the isolate with the source serum has not been attempted.

Results of a neutralization test of two isolates from acute IH serum by their homologous convalescent antiserum is given in Table 9. Again with the "Pom" serum the prozone-like phenomenon was demonstrated but in this case the serum controls are satisfactory. A two month convalescent serum from one of these patients (Pom) was positive for agent and negative for antibody.[1]

Different patterns of neutralization with 1:40 dilutions of stored reference serums from certain laboratory personnel are shown in Table 10.

are certain similarities in the pattern of these three specimens The last two samples are from acute IH and do not appear to be neutralized completely by any of the serums this however may be purely a matter of virus concentration rather than antigenic constitution On the basis of these results however USA TC_6 (number 16) seems to have shifted from the pattern seen with the same material at the TC passage level (number 2) It is possible that another agent may have been picked up in the intervening tissue culture passages but further work will be required to clarify the point

I will not attempt at this time to review and correlate the findings since much substantiation and additional data are needed On the basis of these data we cannot be sure that the virus of hepatitis is being cultivated but it is not unlikely and certainly further work is indicated However there is one more thing before closing We have been intrigued by the marked cell specificity exhibited by these agents Why of all the cell lines tried in our laboratory and elsewhere has only Detroit 6 given reproducible results and this only under rigid control of the cell line and passage conditions? Possibly in establishing cell lines everyone has in some way been selecting nonsensitive cells If this were the case one must hypothe

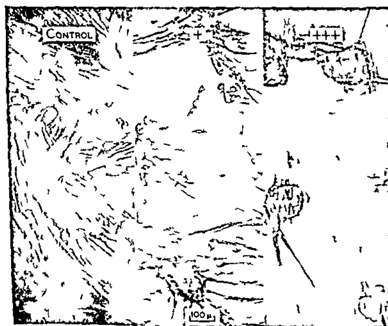


Fig 3

TABLE 10

NEUTRALIZATION TEST ON SERUMS FROM LABORATORY PERSONNEL

Serum 1:40	Agents Tested				Control
	Gar TC4	Kel TC4	Las TC4	Mk TC4	(Serum)
Rh1 (Pre program)	+++	+++	+++	+++	000
Rh2 (Early convalescence)	000	+00	++0	+++	000
FT (History IH)	000	000	000	000	000
PS (Negative history)	++0	++0	000	000	000
HJ (Negative history)	+++	+++	+++	+++	000
Control (CPE)	+++	+++	+++	+++	(Cell 000)

TABLE 11

NEUTRALIZATION PATTERNS OF SEVERAL PASSAGED AGENTS AND ORIGINAL SPECIMENS VS SELECTED HUMAN SERUMS

	Agents Tested	Serums 1:50						CPE Control
		A	B	C	D	E	F	
1	MR1 TC12 Acute IH serum	0	±	0	0	0	±	+
2	USA TC2 Acute IH stool	0	0	0	0	0	±	+
3	Har TC3 Convalescent IH serum	0	0	0	0	0	±	+
4	Lo C TC3 Plasma producing SH	0	0	0	0	0	±	+
5	Lo C Original plasma producing SH	0	0	0	0	0	+	+
6	M Original postinoculation SH (A 70)	±	±	0	0	0	+	+
7	M TC Postinoculation SH (A 70)	±	+	0	0	±	±	+
8	M TC3 Posticteric SH (A 65)	±	±	0	0	0	±	+
9	Pom TC3 Acute IH serum	±	0	0	+	±	+	+
10	Pom TC Acute IH serum	±	+	0	+	0	±	+
11	Pom convalescent TC3 convalescent IH serum	+	+	0	+	0	±	+
12	Tor TC2 IH stool	+	0	±	0	0	±	+
13	Pj 3 Original IH stool	+	+	+	0	+	+	+
14	Detroit 6 control Phila	+	±	±	0	+	+	+
15	Sal TC IH Acute serum	±	±	+	±	±	+	+
16	USA TC6 Acute IH stool	+	+	±	+	±	±	+
	Serum controls	0	0	0	0	0	0	0 Cell

first two antigens are typical isolates from acute IH specimens and Har is the isolate from the convalescent serum shown in Table 8. 4 and 5 are the fourth tissue culture passage of a plasma sample known to produce SH and the original plasma sample from which it was isolated. The next three samples are from Dr Murray's studies on SH. They seem closely related but somewhat distinct from the others. Agents 9, 10 and 11 are an early and later passage of Pom and an isolate derived from an early convalescent serum obtained from the same patient. The next group 12 through 14 are all derived from materials from Dr Henle's laboratory. Note that 14 is from Detroit 6 controls passed in Philadelphia. There

IO

*Studies on the Hepatitis Virus**

SVEN GARD M D and KNUT ALIN M D
(Stockholm Sweden)

A systematic study of the etiology of infectious hepatitis has been carried on at the Department of Virus Research in Stockholm for more than five years The interest has mainly been focused on the possibility of isolation of a transmissible agent in cultures of human embryonic tissues The work has not yet yielded unequivocal or conclusive results In spite of this a summary of the observations made may be justified

The tissue culture experiments were carried out by Dr Knut Alin who will later publish a detailed account of his work Systematic attempts at isolation of a virus in cultures of different human embryonic tissues were initiated in 1952 In his first experiments Alin worked with lung fibroblasts in plasma clot cultures A dozen stool samples and a few serum samples all collected in the acute phase of the disease were examined On no occasion was any cytopathogenic effort of the inocula observed After this experience Alin turned his attention to liver cell cultures Two slightly different culture techniques have been applied

In earlier experiments we had encountered certain difficulties in attempts at trypsinization of embryonic tissue Very often the addition of trypsin to minced tissue caused a congelation of the material or if the cells were obtained in suspension they usually exhibited a strong tendency to agglutinate and their capacity of growth was often poor For the preparation of glass grown cultures we had found the following technique useful A weighed amount of tissue was finely minced and suspended in the fluid medium Coarse matter was removed by passage through a fine nylon mesh The suspension was then diluted to a standard density and distributed in tissue culture tubes which were kept stationary in a horizontal position at 37 C After to 4 days the cells had usually become attached to the glass wall and were rapidly growing Not only epithelial but also glass grown fibroblast cultures were easily obtained

In his first series of experiments Alin employed this technique Embryos of an age of approximately 4 months were used throughout The fluid medium consisted of bovine amniotic fluid or Parker 199 with addition

* The work here described was aided by grants from the Karolinska Institute Research Fund

cate two factors first sensitive cells must be of human origin and for some reason require human serum for growth second as we have demonstrated many human serums must contain virus like agents that could inhibit the growth of the sensitive cell component of the culture After a relatively few passages in such serum only nonsensitive cells would remain to be propagated Thus the maintenance of sensitivity of the Detroit 6 culture would be due to a fortuitous combination of circumstances at isolation and in early passages Moreover if this hypothesis is correct one should be able to utilize sensitive Detroit 6 cells as a tool for pretesting and selecting negative serums for use in establishing new cell lines from human tissues When established such cells should retain their sensitivity Figure 3 shows the results of such a procedure In addition to this human embryonic lung culture a liver culture prepared in the same way is also sensitive The degenerative changes are typical and we trust that sensitivity will be maintained through continuing passages with good serum of these normal human cells One naturally wonders if the problem illustrated here might not well be the difficulty standing in the way of the cultivation of a number of other important viruses

Addendum

A further passage of the specimens supplied by Dr Melnick has now been completed (Table 4) Samples 3733 and 3735 are definitely positive and the agents isolated from the other acute specimens passed readily Third passage of 3953 however remained negative All of the acute specimens have now been shown to be positive making the result even more suggestive

Acknowledgements

The data presented here are the results of the combined efforts of the Virus and Tissue Culture Research Sections and the wholehearted cooperation of the staff is gratefully acknowledged Also we are indebted to all of those who have supplied the clinical specimens which have made this study possible

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tion was limited to 15 to 25 minutes From such suspensions a good growth of liver epithelium was obtained while fibroblasts were few and poorly growing Lactalbumin hydrolyzate or Parker 199 was used and cultures were initiated with 10 per cent human serum

So far 15 stool samples and 9 serums have been examined with this technique in altogether 28 attempts at isolation The results have been largely similar to those in the first series Degeneration of primary cultures was observed on 12 occasions it was reproduced in 11 of 1 attempted first passages in 3 of 11 second passages and then petered out The following description may serve to illustrate the findings

> Patient S B male 53 years old First symptoms December 30th jaundice January 6th blood sample collected January ,th A set of five culture tubes inoculated with 0 1 ml serum showed degeneration on the fifth day after inoculation On passage signs of degeneration were observed on the seventh day on the ninth day 3 plus degeneration was recorded in one tube and slight degeneration in two more In the next passage slight degeneration developed in two tubes on the fourteenth to eighteenth day In the third passage one tube showed 3 plus degeneration on the fourteenth day and one more became degenerated on the eighteenth In the fourth passage one tube showed some degeneration on the sixth day on the eleventh day both inoculated and uninoculated cultures were showing signs of degeneration A further subculture remained completely negative

In most cases tissue culture fluid harvested from degenerated cultures served as inocula for subcultures Some attempts were made to obtain more regular results by using triturated tissue instead This procedure did not improve the results however

The most marked effect of positive inocula was inhibition of cell growth In addition degenerative changes were observed such as swelling granulation and loss of structural details and staining qualities Sometimes the cells coalesced to form multinuclear syncytial masses None of these changes had any specific character however the same phenomena were observed in uninoculated cultures when kept for a sufficiently long period of time

The evaluation of these observations is difficult A carry over and gradual dilution of a toxic substance has of course to be sincerely considered This would be a natural explanation as far as stool specimens are concerned It seems somewhat less plausible in the case of serum samples however since a large number of serums from healthy persons mainly blood donors have failed to produce similar changes Still an adverse effect of icteric serum must be borne in mind Adequate controls with material from cases of jaundice of nonviral etiology have not yet been carried out

Alin has considered the possibility that the more differentiated cells from adult livers might be more suitable for demonstration of a cytopatho

of soy bean inhibitor Approximately two-thirds of the cultures were grown in rooster plasma clot as roller tubes the remainder were glass grown and kept stationary The cultures were initiated with addition of 40 per cent human serum or 10 per cent horse serum After 3 to 6 days incubation they were washed with and further maintained in serum free fluid with change of medium every 4 days

In this series 3 stools and 1 serums were examined Specimens were collected during the period August 1954–November 1955 from patients hospitalized under the diagnosis of acute infectious hepatitis They were stored at − 0 C. or −60 C. until used Cultures were inoculated in connection with the first change of medium and were then observed for at least 1 days or as long as uninoculated control cultures did not show signs of degeneration Some cultures could be maintained for up to 3 weeks

In roughly half of the inoculated cultures degeneration of the epithelium was observed at a time — usually 5 to 10 days after inoculation — when the control tubes still remained normal In such cases passages were performed and continued as long as degeneration appeared in the subcultures The changes observed in the initial cultures could not regularly be reproduced however Sometimes the phenomenon was repeated on subculturing but the changes tended to occur later and to be less pronounced in each subsequent passage

However one transmissible agent was established in this series A stool stample collected on the fifth day of illness from a typical case of infectious hepatitis produced degeneration in 3 out of 4 sets of cultures Only one of these gave rise to similar changes in subcultures In further passages the cytopathogenicity increased gradually the incubation period decreased and finally not only epithelial cells but also fibroblasts were attacked

In complement fixation tests serum from the patient has consistently yielded negative results even late in the convalescence Several other convalescent serums as well as a number of serums from normal adults on the other hand fix complement with this strain No systematic attempts at typing within the Coxsackie and Echo groups have as yet been carried out The identity origin and significance of this agent are thus still obscure

The tissue culture technique used in the first series of experiments was not entirely satisfactory Liver epithelium was never obtained in pure culture fibroblasts were always present and tended to overgrow or inhibit the growth of the liver cells When therefore an outbreak of about 600 cases of hepatitis in December 1955 provided abundant material for further experimentation Alin decided to change his methods It was found that satisfactory cell suspensions could be obtained if trypsiniza

II

*The Growth of Organ Cultures of Liver and Their Use in the Study of Hepatitis**

FREDERICK B BANG MD

(Baltimore Maryland)

When human embryonic liver tissue is explanted into roller tube cultures with human cord serum there eventually results a mixed outgrowth of fibroblast hematopoietic elements macrophages and even cords of epithelial cells These are arranged as liver cells might be expected to be if made to live within two dimensions However the fibroblastic or other elements of the culture eventually overwhelm the central organized portion A procedure such as trypsinization which frees the epithelial cells at the start may yield a higher proportion of liver parenchyma but also usually yields a layer of disorganized separated cells appearing in sheets often monolayer in thickness

Those types of tissue culture which favor the continued maintenance and growth of cells in an organized state so that they preserve their morphological relationship one with another are usually called organ cultures When the morphological criteria include the presence of cilia and the secretion of mucus by epithelial cells it is obvious that a large part of the functional activities have also been preserved There is disagreement among organ culturists as to whether active mitosis helps or hinders the continued organization of these cell colonies although total cell mass may not always increase but mitosis is certainly usually present

Probably one of the requisites of a successful organ culture is that the original group of cells be kept in some close relationship to each other — and this preserves their organization

Organ cultures have been used predominantly to study the development of morphological units of the whole and also used by embryologists who were interested in understanding the potentialities of various specific tissues of the embryo They have also been successfully used to study the interrelationships of endocrine glands including the ovarian testis relationship However until recently they have not been used to study the basic problems of specificity of infection During the past year I have been studying with Dr Niven at the National Institute of Medical Re

* Supported by the Armed Services Epidemiological Board.

genic effect of the hypothetical agent He has therefore attempted to establish cultures from material obtained by liver biopsies In most cases these efforts have failed as a matter of fact only one successful experiment has been recorded so far For this reason no systematic study of the question could be carried out

Lately we have tried a new approach to the problem of cultivating the virus in vitro The above mentioned outbreak of some 600 cases of infectious hepatitis could be traced to oysters as vehicles of the infection On the basis of the observations made it must be sincerely considered whether an actual multiplication of the virus may not have taken place in the infected oysters As a first attempt to obtain an answer to this question we have tried to establish oyster tissue in culture This has proved to be much more difficult than originally expected On one or two occasions an outgrowth of sheets of extremely small epithelium like cells was obtained The results could not be reproduced however and it is still an open question whether or not the growth really consisted of oyster tissue

As a preparation for work on live oysters and also for purposes of identification of any suspected agent presumably propagating in liver tissue cultures we have carried out some experiments with the fluorescent antibody technique A fluorescent rabbit anti gamma globulin was prepared It reacts in high titers with gamma globulin and gives a clear gel precipitation pattern with its homologous antigen In tests with hepatitic liver tissue a rather diffuse fluorescence of the liver cells was observed In controls not pretreated with gamma globulin a similar picture was observed So far we have not been able to detect any indication of a specific reaction

To summarize the search for a method to establish the agent of infectious hepatitis in tissue culture has proved rather frustrating It would seem however that the observations here reported merit some further examination

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search the use of this type of culture in various infections This work will be reported elsewhere but we may say that a variety of viruses do produce their specific lesions in culture of human and animal tissues

Since we have failed during the past six years to get human hepatitis virus to destroy either embryonic or malignant human cells in tissue culture we have started to explore the use of organ cultures of human liver in this relation Although sufficient work has not been done to tell whether such liver cells will succumb to infection we have been able to maintain these bits of embryonic liver in cultures of chicken plasma chick embryo extract and have with chick liver been able to destroy such organ cultures with the virus of duck hepatitis Thus a method of growing bits of human liver as liver is at hand and a method of study of an analogous infection in these cultures is available If eventually the viruses of human hepatitis destroy separated human cells in vitro or produce recognizable infection of other animals it will still be of interest to study the pathogenesis of infection in organ cultures for they lack vascular circulation and are not dependent upon an intact host animal for survival Probably much if not all of the host's antibody response is absent and yet they have many of the characteristics of the organ from which they are derived

The continued stubborn resistance of the problem of hepatitis means to us simply that it would be of value to have more people study it and with that in mind we make no apology for telling you of our present incomplete work

Human Embryonic Liver Embryos varying in size from a few centimeters to perhaps seven months of development were used The majority of these were obtained within several hours of the operation and were used shortly thereafter Bits of tissue cut with sharp knives were placed on a rayon (cellulose acetate) net which in turn was placed on a chicken plasma clot in a watch glass The entire culture was kept in a moist chamber and handled essentially according to Strangeways Laboratory techniques The cultures were incubated at about 36 C without CO They were transferred one to two times per week

Within the first two days the rough irregular edge of the piece of explant was converted into a smooth even partially translucent edge If the tissue had been placed on a clot the piece soon flattened sent out wandering cells which clung to the strands of rayon and cemented the tissue to the net so that transfers to subsequent media which often included washing of the tissue with balanced saline were facilitated Other cultures were maintained on a liquid medium obtained by grinding a plasma embryo extract clot in a glass grinder These cultures rounded up and often had a more translucent edge than their colleagues on the firm clot However a comparison of the appearance of these cultures in section shows that this

may be misleading for this rounding of the entire raised culture occurred despite the continued presence of large necrotic areas in the center of the culture The present sectioned material indicates that a larger proportion of healthy organized cells was obtained by growth on firm plasma clots

The embryonic liver cultures are organized into two major components which are easily studied in section They are of course the liver cells themselves and the hematopoietic system The entire culture is covered with a thin layer of epithelial cell and below this are cords of liver cells mixed with macrophages, which continue their activity of swallowing red cells and converting them into pigment The lakes of hematopoiesis which also contain formed red cells are usually bordered by thin endothelial cells, giving the entire culture a picture much like capillaries

We have not maintained these cultures beyond three weeks but have as yet made no consistent effort to do so with liver cultures Martinovitch records the disappearance of the necrotic central area and the reorganization of the young rat pituitary during a period of two months of cultivation by a slightly different method It is difficult to generalize about the appearance of the culture as an indication of healthiness but coherence and smoothness are characteristics of healthy cultures There is however as yet no substitute for adequate sectioning For example buried within a poorly nourished culture one may find islands of liver cells

We have only recently begun to study the growth and appearance of adult human liver in such cultures We have no data on the possible value of using mammalian serums or the relative value of different types of embryo extract

We have studied similar cultures of chick embryo liver so that we might follow the effect of a duck hepatitis virus on these cells These grow more rapidly in organized cultures and often have a deeper transparent periphery Again the two types of media yielded somewhat different results and the correct interpretation was obtained by study of sections

The virus of duck hepatitis was described by Fabricant and Levine about six years ago * This virus may kill young chick embryos with a generalized hemorrhagic reaction which is most marked in the feather follicles Older embryos show the results of liver destruction in that they are either extremely anemic with gross destruction of the liver or the live intestine and even the allantoic fluid may turn green presumably with the breakdown products of hemoglobin — perhaps biliverdin

Electron microscopy of such infected livers shows a general distortion of the cellular architecture but nuclei mitochondria endoplasmic reticulum and the ribonucleic acid protein granules persist though not uniformly distributed throughout the cell No virus particles have been seen

* Dr Asplin of the Agriculture Research Council of Weybridge was kind enough to show me some of his early work and thus stimulated our study of this virus

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DESIGNATED DISCUSSION

THOMAS H WELLER M D (Boston Massachusetts) It had been my intent to initiate this discussion by dwelling for a few minutes on some of the current trends in tissue culture as they apply to today s subject but because Dr McLean has so nicely brought out some of the problems encountered in working with cell lines we will go on to the second item that I wish to mention

I would like to comment briefly on a virus that of so called cytomegalic inclusion disease which we have been studying Apparently similar agents have been isolated in St Louis by Smith and by Rowe and co workers in Bethesda

Our interest in this entity began almost two years ago when we were supplied with liver biopsy material from a three month-old infant with evidence of obstructive jaundice hepatosplenomegaly and microcephaly Histopathologic examination of the biopsy showed diffuse alteration of architecture with extensive bile stasis degenerate hepatic cells with scattered multinucleate giant cells as well as focal areas of erythrocytopoiesis and a portal round cell inflammatory infiltrate

Inoculation of the liver biopsy material into cultures of human embryonic foreskin tissues resulted in focal changes in fibroblastic cells characterized by the deposition of brown pigment Stained preparations revealed the presence of large intranuclear inclusions Careful search of serial sections of the original liver biopsy then demonstrated very rare cytomegalic cells with intranuclear inclusions

The development of an in vitro neutralization test has yielded data indicating that specific neutralizing antibodies occur frequently in infants with cytomegalic disease as well as in a significant percentage of normal adults

More recently another strain of virus has been isolated from liver biopsy material of a three week old infant showing jaundice and hepatosplenomegaly The biopsy from the second patient also showed a picture compatible with that described by Craig and Landing under the term neonatal hepatitis In this instance no cytomegalic cells could be demonstrated in the liver section on prolonged search From the urine of this patient virus was isolated on three occasions between the fourteenth day and the ninety first day of life Even more recently from two additional infants with signs of obstructive jaundice similar viruses have been isolated in vitro using urine as the inoculum

In view of the apparent ubiquitous distribution of the so called cytomegalic or salivary gland viruses the question of the possible etiologic role in neonatal hepatitis of these newly isolated agents must be approached with caution Isolation of virus from the damaged liver does not

We have shown that this virus will grow in chick embryo liver organ cultures with gross destruction which is produced by the virus Thus despite the lack of detailed cellular study it does seem possible to follow the effect of the virus in organized tissue cultures Yet before organ cultures may come into full use better methods of determining the effect of viruses on these cells are needed

In summary human and chick embryonic liver when grown in organ culture retain a large degree of their original organization which includes the formation of cords of liver cells hematopoiesis and endothelial lined vascular areas Although such cultures are best studied by histological sectioning gross changes caused by destructive viruses may be observed directly

to a mother whose serum on two occasions in over a 30 month interval had produced hepatitis in volunteers The infant developed the disease whatever the hepatitis was — and we believe it was probably viral hepatitis — after about thirteen and one half months and died at 18 months The serum of this child at 6 months produced hepatitis with jaundice in some volunteers and hepatitis without jaundice in other volunteers

This suggested to us resulting presumptive evidence We fully agree that serum hepatitis had occurred in the mother who had negative liver function tests throughout on all occasions on which she was tested and perhaps she had transmitted the disease to her infant through the placenta

I think there is one other possibility in such a case the virus of hepatitis might have been transmitted by means of the spermatozoa from the father We particularly selected this case because it was a case of Cesarean section delivery The infant had no contact with the birth canal so we could rule out virus in the intestinal tract and it also had no contact with the mother as far as milk was concerned so it suggested either spermatozoa or transplacental infection

Ralph W Brauer Ph D (San Francisco California) I feel a little timid about asking questions of a clinical audience of such distinction but it seems to me we have heard a great deal about neonatal hepatitis and yet I am not aware of having seen or having heard anyone discuss hepatitis in the unborn

Does this mean that the hepatitis is latent and becomes a manifest entity only when the liver is subjected to the rather abrupt changes both circulatory and metabolic that are associated with bringing the poor little character out into the world or does this mean that the infection does take place only after the infant is brought out and that it then becomes manifest?

Chairman Dalldorf There have always been gross deficiencies in our knowledge of the pathology of the unborn for very obvious reasons but that has been a serious and important gap in that knowledge

Dr Prauer You would expect such infants to come out stillborn would you not? The liver does help in survival

Chairman Dalldorf It is a provocative question and I will be glad to have someone comment on it.

G A Martini M D (Hamburg Germany) Since Dr MacCallum mentioned our findings I want to summarize some of our data

necessarily indicate a causal relationship, yet it seems not improbable that additional work may incriminate the cytomegalic virus as one of a spectrum of agents capable of producing a viral hepatitis in man.

GENERAL DISCUSSION

JOSEPH STOKES, JR., M.D. (Philadelphia, Pennsylvania): I think the rest of the afternoon will be taken up with consideration of many of the problems that Dr. MacCallum brought up. It may be worthwhile mentioning two things that he discussed, in which we have been particularly interested.

One was the question of whether gamma globulin prevents actual infectious disease — whether it prevents the occurrence of apparent or inapparent disease.

After a good many studies of gamma globulin, which suggested that there was not the prevention of infection, a study was carried out in our laboratories by Drake and Main, in which about 156 children were injected with gamma globulin in the same cottages in an institution for retarded females, and approximately 70 remained as controls.

Over a period of 15 weeks, 8 liver function tests were carried out on each of these children every week. The number of cases of jaundice in the injected individuals ceased after the first week, with one exception. On the other hand, in the control children over 40 cases of jaundice occurred, and about 85 cases of hepatitis without jaundice. Hepatitis without jaundice was estimated as occurring when there were 5 positive liver function tests.

The percentage of hepatitis without jaundice in the injected group approached the total number of cases that had occurred in the control group of both jaundiced hepatitis and hepatitis without jaundice. This fairly clearly indicated that the gamma globulin had not protected the injected individuals from infection, since the two incidence rates approached each other in the two groups.

After these studies and others that we have carried out, we do not believe that gamma globulin protects the individual from infection. However, it does protect the individual from serious disease with jaundice, and it may also protect him from chronic active hepatitis, which has been suggested by the gamma globulin studies. This naturally becomes a question of postimmunization, which will be discussed later.

I should like to say something about Dr. MacCallum's mentioning of neonatal hepatitis. I think Dr. Weller has indicated, as Dr. MacCallum did also, that once you have caught it, neonatal hepatitis is not viral hepatitis. We have had a great many cases of hepatitis in the newborn, but in only one instance have we felt that we might have had a case of viral hepatitis, probably serum hepatitis, in this case. That was in a child born

in pregnant women pertained only to the epidemic in Delhi In the figures I have given in Dr Allen s report, which probably will be read later at this conference the incidence of hepatitis was very great in pregnant women and the mortality in particular was high The general mortality as given in his report was 0 09 The mortality in pregnant women was 10 per cent if no abortion was induced

CHAIRMAN DALLDORF I am surprised that there has been no discussion of Dr McLean s paper because I know there are a number of people here who have been working with the cell system that he reported

I was told that Dr Hok is with us and has had experience that I haven t heard of before If that is the case and if he would like to report on his observations I would be very happy to recognize him

KAROL A HOK M D (Berkeley California) I would like to mention our own attempts at isolating the agent of hepatitis from serum specimens

The agent was carried through six passages with reproducible results and sixteen individual repeat experiments were carried out The results were reproducible Further passages had to be discontinued because the particular serum in which these cells were grown was exhausted

I would like to mention that this particular sequence of events took place in cells grown in one particular serum No 3 Sublines of these cells grown in other individual human serums failed to reproduce this particular effect Therefore we were forced to accept the notion that human serums had to be so infected in order to find a suitable serum for the growth of the cells and subsequent infection by the virus Incidentally the original serum specimen was diluted to 1 to 20 000 by means of six passages At the same time a 1 to 100 dilution of the original specimen repeatedly failed to infect the cells

I wish to mention just one complication which Dr McLean also pointed out The gross similarity between normal and infected cultures of Detroit 6 cells is evident yet on close examination we believe that it is possible to pick out differences There seems to be a difference in the degree of granulation and the thickness of the clump itself This fortunately does not happen very often This is our only successful passage of the agent from a hepatitis case The others have not been so consistently classic so that is as far as we have gotten

C STULBERG, Ph D (Detroit Michigan) I would like to make a few comments regarding Dr McLean s paper

The culture of Parke Davis s Detroit 6 maintained for four months in our laboratory in Eagle s basal medium plus 20 per cent human cord or adult serums consistently undergoes a cyclic spontaneous degeneration

I was surprised to hear from Dr. Smetana that the incidence of hepatitis in pregnant women has been higher than in other groups. We were able to collect our data after the war from Hamburg, where hepatitis was notifiable. We had an incidence of 7 cases in 10 000 in the general population as compared with 4 in 10 000 in pregnant women.

We saw 57 women in a 5 year period, and we have seen about 70 cases altogether now. In no case was there evidence that the virus passed through the placenta. In 5 cases histological studies of the liver of the babies showed no evidence of pathology either. There was a higher incidence of abortion and miscarriage.

In several series from Copenhagen, Vienna, Israel and Hamburg, with about 50 cases of proven hepatitis in pregnant women, no case has been found so far in which the child showed certain evidence of hepatitis. We made a follow-up investigation and found that 1 women had one or two more normal pregnancies later. None of the children was affected.

ANDREW SASS-KORTSAK, M.D. (Toronto, Canada): We have been observing a newborn baby who was born to a mother suffering from hepatitis during the delivery. The delivery, in fact, may have been precipitated by the mother's disease. She was in the early stage of her jaundice.

The reason I think this case may be worthwhile reporting here is that careful history disclosed that three weeks before the mother's illness she was looking after a boarder who had a clinically typical infectious hepatitis. Thus it seems this mother was suffering from hepatitis due to infectious hepatitis virus.

My second remark is in connection with Dr. MacCallum's paper. First, full galactosemia and hemolytic disease are obstructive types of jaundice in early infancy. However, I do not think that this accounts for all cases of so-called obstructive jaundice of unknown etiology in early infancy.

In our series—and I believe in Boston and other places as well—galactosemia and hemolytic disease in the newborn have been very adequately excluded as causes, and still there are cases of obstructive jaundice which may be due to causes that Dr. Weller has been talking about.

I would also like to mention that in our series the detailed liver function studies have grouped our patients into two distinct brackets. There was one group which conformed to the Boston reports, having perfectly normal parenchymal liver function tests. However, we have seen another group in which we had positive cephalin flocculation, decreased albumin levels and, generally speaking, changes typical of virus hepatitis in adults. One of these cases was part of that family incidence. There were three babies involved in that family.

HANS F. SMETANA, M.D. (Delhi, India): The reference I made to hepatitis

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*Epidemiology of Infectious Hepatitis**

JOHN R PAUL MD
(New Haven Connecticut)

In accepting this assignment I am conscious of the fact that there is much about viral hepatitis which remains obscure and it may be too early yet to attempt to document its epidemiology My report is therefore presented at the considerable risk of being premature and inadequate ** Furthermore it will be limited to a discussion of that form of hepatitis caused by virus A (or IH) which is now regarded as the etiologic agent of *infectious* hepatitis [1] and I shall not attempt to cope with the puzzling questions which concern the relationship with serum hepatitis due to virus B (or SH) Furthermore the review will deal more with generalities than with specific aspects Special aspects of the epidemiology of both serum and infectious hepatitis will be taken up by other participants

Prevalence Apparently the incidence of hepatitis has been much on the increase since World War II not only in the United States but elsewhere even in Soviet Russia During this short period new concepts have arisen regarding pathogenesis and nomenclature In some measure the increase in viral hepatitis rates reflects therefore a rise in interest in this disease as an example of nosography based on the significance of a new name For as long as viral hepatitis masqueraded under the names of acute catarrhal jaundice (regarded as a common mild infection) or acute yellow atrophy of the liver (regarded as a rare although very serious disease) it occupied a back seat But now with the introduction of the term viral hepatitis all this has been changed and one has to deal with a common disease which in adults has moderately serious implications and whose new name carries the threat of contagion This has created a great increase in interest It is difficult to determine therefore how much of the increase

* Much of the work on which this review is based is sponsored by the Commission on Viral Infections Armed Forces Epidemiological Board

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similar to the cultures that Dr Holt and Dr McLean have shown which were affected by these agents

Our original strain does not show and never has shown this effect although maintained under identical conditions Therefore there may be differences in the various Detroit lines as Dr McLean has suggested and it may be that our own cell line maintained in our own laboratory may not be sensitive

Secondly I want to correct several designations given to the various Detroit strains by Dr McLean They are not Stulberg strains They were isolated and established by Dr Lawrence Berman in his laboratory at Wayne State University in collaboration with us at the Child Research Center

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*Epidemiology of Infectious Hepatitis**

JOHN R PAUL, M D

(New Haven Connecticut)

In accepting this assignment I am conscious of the fact that there is much about viral hepatitis which remains obscure and it may be too early yet to attempt to document its epidemiology My report is therefore presented at the considerable risk of being premature and inadequate ** Furthermore it will be limited to a discussion of that form of hepatitis caused by virus A (or IH) which is now regarded as the etiologic agent of *infectious* hepatitis [1] and I shall not attempt to cope with the puzzling questions which concern the relationship with serum hepatitis due to virus B (or SH) Furthermore the review will deal more with generalities than with specific aspects Special aspects of the epidemiology of both serum and infectious hepatitis will be taken up by other participants

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in incidence is real and whether new strains of hepatitis virus were spread throughout the world as has been suspected by invading or returning troops during and after World War II

In the case of serum hepatitis which is almost undistinguishable clinically from infectious hepatitis there is good reason to believe that the incidence rate is actually increasing in this country for the mounting frequency of the use of blood transfusions, blood products and many types of inoculations or parenteral therapy all offer a growing opportunity for the inadvertent transmission of serum hepatitis virus In infectious hepatitis there is also a viremic stage and it may of course also be transmitted occasionally by parenteral methods In this connection I should mention a feature that has long impressed me although I have no data to demonstrate it This is that wherever infectious hepatitis is prevalent there one also finds often unexpectedly cases of serum hepatitis

With orally transmitted infectious hepatitis on the other hand it is something of a guess as to whether this form is on the increase or not. Through the kindness of Dr T H Ingalls of the Department of Epidemiology of the Harvard School of Public Health I have been privileged to review data which may have some bearing on this in New England concerning the prevalence of acute hepatitis and catarrhal jaundice as it has occurred over a 50-year period in a large private boarding school in Massachusetts In this institutional population the minor and the serious illnesses have been constantly recorded by the school physicians from these records one is in a fair position to compare the chronological incidence of cases in this particular group of boys 1 to 19 years of age in different decades The data indicate that there was no statistical difference in the total number of cases of jaundice or hepatitis in the school population during the first and second quarters of this century However between 1900 and 19 4 the cases labeled catarrhal jaundice were largely sporadic whereas an epidemic in 1945 accounted for the great majority of cases in the 19 5–1950 period

Geographic Distribution In spite of inadequate information there is little question that so called viral hepatitis is a common mid twentieth century disease and is very widespread Geographically it probably occurs in nearly all inhabited areas of the globe although there are certain regions with an established record of high endemic prevalence of which the Far East and the Mediterranean littoral are examples Over the past 150 years severe epidemics have occurred repeatedly in the latter area among troops from Europe Australia and the North American continent which have been brought into this endemic area — a point which Dr Hill made in his paper

In Scandinavian countries long term information on the incidence of

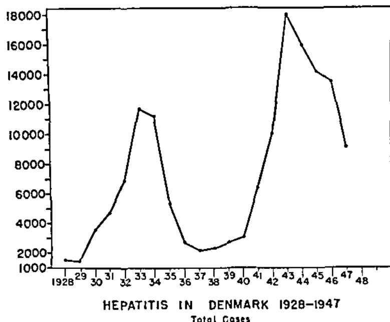


FIGURE 1 Fluctuation in the numbers of cases of hepatitis over a 20-year period (Data collected by Havens from the Danish Ministry of Health)

hepatitis has been more complete than elsewhere This disease has been notifiable in Denmark for instance since 1928 (see Figure 1) Here it will be seen that in keeping with the experience of some other countries in temperate climates in which sanitary facilities are standard or above the incidence has varied greatly with waves of high incidence covering several years notably those of the war years when that country was occupied by German troops How representative of northern Europe this picture may be is not known In any event it is unlikely that the Scandinavian incidence picture would be similar to that of a tropical country or one with substandard conditions of environmental sanitation

In the United States as a whole the reporting of cases during the past decade has been irregular and often on a voluntary basis This situation is now being rectified but at present it would be difficult to say what the differential incidence rates in this country on a state wide basis might be

Transmission Most present evidence indicates that infectious hepatitis is usually spread through some form of person to person association although explosive water borne food borne and milk borne epidemics have been described Nevertheless man represents the only known source and

reservoir of the infection As yet no known extrahuman host in the form of an animal bird or arthropod has been discovered in which hepatitis viruses are known to multiply or survive, although there are of course channels such as water in which this hardy virus can survive and be spread and the possibility of mechanical transmission by a biting insect has not been adequately investigated

There is good evidence to indicate that the intestinal oral circuit plays a dominant part in this natural person to person spread Such evidence is based upon experimental evidence with human volunteers which indicates the frequency with which hepatitis virus (IH) can be demonstrated in human feces during acute stages of the disease and the ease with which the experimental disease may be produced by feeding such material to susceptible hosts Although some epidemiologic observations point to a respiratory mode of spread none of the experiments on this has been sufficiently satisfactory (due to technical or other reasons) for definite conclusions to be drawn from the results This intestinal oral spread does not mean that water borne epidemics are the rule in fact quite the contrary in this day and age of good environmental sanitary arrangements Personal and close contact with infective people as is the situation with poliomyelitis probably accounts for a great majority of orally acquired cases of infectious hepatitis

As to the duration of the period of infectivity in the usual acute case this is not known but it is usually considered that the virus is excreted in the feces during the active phase of the disease and perhaps for some days afterwards In infants however the carrier state may be protracted for months [3]

Evidence to substantiate the fact that the intestinal oral circuit is involved is based upon a number of epidemiologic features which include (1) The seasonal trends which the incidence of this disease exhibits with a rise in incidence in summer and early fall which is a characteristic feature of a variety of enteric infections (Figure 2) (2) the observation that epidemics of infectious hepatitis are often preceded by an acute outbreak of gastroenteritis — an experience repeatedly seen in North Africa during World War II An example of this has been recently reported by Tucker Owen and Farrel in the form of a water borne epidemic which occurred at a church camp in Tennessee in 1952 [4] This and other similar situations have suggested that both infections — the acute gastroenteritis and infectious hepatitis — were related to the same source (3) Military observations record that hepatitis incidence is highest when and where the sanitation of camps is poor Civilian evidence also testifies to the frequency with which visitors acquire hepatitis in tropical or semitropical areas where poor sanitary facilities exist and to the fact that in institutions for mentally deficient individuals the hepatitis rates have been higher in those

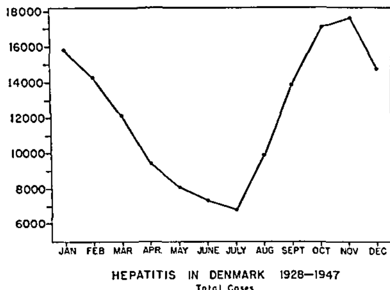


FIGURE 2 Seasonal trend from data covering a 20-year period (Data collected by Havens from the Danish Ministry of Health)

buildings housing individuals with the lowest mental capacity and therefore in which the poorest sanitation can be maintained

Nevertheless it should again be emphasized that as viral hepatitis is easily spread by intimate association among people epidemics of this disease may and do occur frequently in institutions dwelling houses or apartment houses where sanitary facilities are far from substandard [5] and they often occur in the best regulated families Within some of these communities it may well be that a large unsuspected source of virus comes into existence in the form of inapparent juvenile cases and under these circumstances it is small wonder that the virus spreads readily to all the susceptibles in the exposed population group

Types of Epidemics

(1) *Water borne* Reference has already been made to the explosive water borne epidemic of which there are now many good examples [4, 6] Prominent among them is the one at a children s camp observed by Neefe and Stokes [7] A notable recent example of a huge water borne urban epidemic of infectious hepatitis is the one which occurred in the city of Delhi India which Dr Melnick describes in this symposium

(2) *Food Handlers and the Spread of Hepatitis* It is more than likely that food handlers in the various infectious stages of subclinical or inap

parent hepatitis plays an important part in the spread of IH virus This has also been documented in military experience

Institutional Outbreaks These are common in orphan asylums boarding schools and notoriously so in mental institutions In some large mental institutions (with a population of 000 or more) hepatitis and salmonellosis may become endemic and over the course of several years cases may continue to appear in large or small numbers each month both in inmates and in attendants (See the report by Dr Ward and Dr Krugman on Endemic Viral Hepatitis in an Institution) This situation has been ascribed to the bowel habits of mentally deficient inmates, be they children or adults and to the great difficulties of maintaining adequate standards of environmental sanitation even in the best run institutions

Family Epidemics These are so common and well known that they hardly deserve mention In a family consisting of five or more members a usual story is that one child contracts a mild case of jaundice first and not much is made of it A month later another child comes down with it or perhaps a parent and in the latter instance a great deal of attention is paid to the adult case after still another month a tertiary case may appear This rather long interval between cases offers an opportunity to apply the use of gamma globulin as a prophylactic measure in such situations.

Age Distribution Under most circumstances and in areas where hepatitis is common this disease is essentially one of childhood Indeed as a general statement children are said to account for 65 per cent of the cases (Figure 3) but it is obvious that this may vary considerably under different environmental conditions Thus the peak of the cases shown in Figure 3 may be skewed to the left or right in different places or at different times in the same place The concentration of the disease in juveniles has been responsible for many misconceptions regarding local incidence because juvenile cases are apt to be so mild as to escape recognition and the adult population which is immune yields very few cases Often this has led to a complete unawareness within a community regarding the high local incidence of viral hepatitis This is in keeping with the epidemiologic behavior of other diseases such as poliomyelitis which confer immunity and whose spread among infants is associated with primitive sanitation Within such areas infection and immunity are acquired so early in life that the clinical symptoms are about at the vanishing point and superficially the disease seems therefore to be conspicuous by its absence Its presence only comes to light when visitors enter the endemic area

The mildness of childhood hepatitis has not received the emphasis it deserves particularly as there is no evidence that such mild cases are any

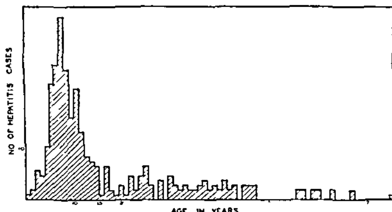


FIGURE 3 The age incidence of epidemic hepatitis in Germany prior to World War II The data are from two outbreaks occurring in Hamburg and Wilhelmsberg respectively (Described by Holm in 1939 and quoted from von Bormann[8])

the less infectious A comparison of symptoms between childhood (6 to 16 years) and adult (military age) cases of hepatitis has been made by Horstmann Havens and Deutsch[9] In the former the total duration of symptoms ranged from to 32 days averaging 11 days and in the latter from 7 to 90 days averaging 30 days

In infants the disease may be still more difficult to recognize mild diarrhea may be the only symptom The observation of Capps and Stokes[3] who have described outbreaks in institutions bears witness to the fact that the clinical disease came to the surface when susceptible adults such as nurses were brought into intimate contact with this reservoir of virus as a result of their care of these infectious but apparently healthy infants In this sense these infants can serve as a *reservoir* of infection Innocent as these infants may appear they may be as dangerous to their own small cosmos as was the Broad Street pump

Sex Distribution From the scanty data available for analysis cases of viral hepatitis seem to be evenly distributed between the two sexes However female parents of the childbearing age may as is also true of poliomyelitis acquire hepatitis at a slightly higher rate than is the case with males of a comparable age[5] It is presumed that the mother s more intimate contact with the children than the father s may be responsible for this effect.

SUMMARY

The epidemiologist s work which is concerned with the behavior of viral hepatitis has just begun One of the major tasks at present is to learn

parent hepatitis play an important part in the spread of IH virus This has also been documented in military experience

Institutional Outbreaks These are common in orphan asylums boarding schools and notoriously so in mental institutions In some large mental institutions (with a population of 000 or more) hepatitis and salmonellosis may become endemic and over the course of several years cases may continue to appear in large or small numbers each month both in inmates and in attendants (See the report by Dr Ward and Dr Krugman on Endemic Viral Hepatitis in an Institution) This situation has been ascribed to the bowel habits of mentally deficient inmates, be they children or adults and to the great difficulties of maintaining adequate standards of environmental sanitation even in the best run institutions

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13

*The Epidemiology of Serum Hepatitis**

W. PAUL HAVENS, JR., M.D.

(Philadelphia, Pennsylvania)

In spite of the sharp limitations imposed by inadequate methods of investigation, certain concepts have emerged about viral hepatitis, its causative agents, their possible ways of spread and maintenance in nature, and the immune responses they evoke. As a result of relatively few experiments in volunteers and numerous clinical and epidemiologic observations, it is now generally believed that at least two forms of immunologically distinct hepatitis exist: (1) the naturally occurring epidemic or sporadic *infectious hepatitis* caused by virus A, and (2) the artificially transmitted *serum hepatitis* caused by virus B. The relationship between these two diseases is obscure, and the arbitrary definition of serum hepatitis on the basis of the route of inoculation of its causative virus adds to the confusion, since virus A may also be found in the blood and transmitted by parenteral inoculation. This doubtless occurs more frequently than is generally suspected, and it is not unfair to say that many aspects of the natural history of the two diseases are at present inextricably bound together.

Not a great deal is known about the epidemiology of serum hepatitis; therefore, it is appropriate to review briefly certain points about etiology and pathogenesis which may have bearing on this aspect. Although virus B is apparently immunologically distinct from virus A, as demonstrated by limited cross immunity tests in volunteers and also by clinical observations, it is not yet known whether this distinction is representative of actually different viruses or of various strains of a single virus with antigenic differences potentiated by as yet undefined influences. The curious apparent dependence of virus B on artificial transmission, its long incubation period, and the contradictory evidence of immunity following infection with it stimulate speculation on the possibility that the family which makes up virus B may represent variants of virus A, which is normally transmitted through the intestinal-oral route, produces disease

* Many of the investigations described herein were sponsored by the Commission on Viral Infections or the Commission on Liver Diseases of the Armed Forces Epidemiological Board and were supported in whole or in part by the Office of the Surgeon General, Department of the Army.

more about its pathogenesis and its relationship to serum hepatitis Personal and close association between people is the most important method of transmission of infectious hepatitis although as the intestinal oral circuit does come into play, water and food represent vehicles which may be significant in the transmission picture Children of school age or infants suffering from subclinical or inapparent infection are very important in the epidemiologic picture because they often serve as carriers or as a reservoir of infection

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under the circumstances of a natural and an artificial way of transmission is self evident

The difficulties encountered in interpreting the immunologic response following hepatitis and the possible relationships between strains of virus are illustrated by a recent report [9] of three apparently self limited attacks of hepatitis that occurred at intervals of one year in a narcotic addict (Figure 1) This man had been receiving intravenous injections in group practice of narcotic administration as often as once a week over a period of three years In view of the relatively solid immunity usually associated with infection with virus A and the poorly defined immune response after infection with virus B the following lines of speculation are valid If one attack of hepatitis was caused by virus A the other two might have been caused by a strain of virus B that evoked insufficient antibody to prevent reinfection or by immunologically different strains of virus B If however virus A was not operative all three attacks might have been caused by a strain or strains of virus B The possibility must also be considered that this patient might be a carrier of virus B in the blood and that under certain circumstances the equilibration between host and virus might be so disturbed as to result in repeated attacks of clinical disease

Certain characteristics of modern civilization and of the behavior of virus B complicate its epidemiology In this regard the widespread use of human blood and its products the unusual resistance of virus to various physical and chemical agents and the frequency and prolonged duration of the carrier state in man are important determining factors Recognition that the role of the carrier as a virus reservoir is therefore important in

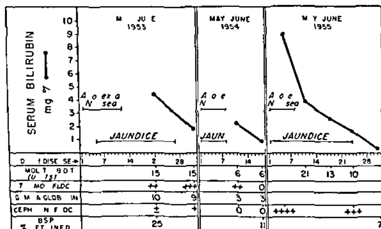


FIGURE Course of disease during 3 separate attacks of viral hepatitis that occurred at intervals of 1 year in a narcotic addict

after a short incubation period and evokes a fairly solid immunity in man. The development of strains of rinderpest virus transmissible only by injection has been cited by MacCallum [2] as a possible analogy in animal disease.

The epidemiology of serum hepatitis is made difficult by the lack of methods to measure the previous immunologic experience with virus B. Little is actually known about the immune response following infection with virus B, and even in the limited data available there is sharp disagreement. Experimentally, Neefe *et al.* and Murray [3] showed that volunteers recovered from serum hepatitis were apparently immune when reinoculated with the homologous strains of virus B several months later. In addition, the fact that a large percentage of persons inoculated with virus B, either as volunteers or as patients receiving injections of contaminated blood or plasma, fail to contract hepatitis indicates that some protection, possibly in the form of antibody, is present. That this is open to question, however, is indicated by the findings of Drake *et al.* [4] who failed to prevent infection with virus B in volunteers simultaneously inoculated with virus and with gamma globulin prepared from persons convalescent three months to three years from serum hepatitis. These latter results are in accord with the apparent failure of normal human gamma globulin to prevent serum hepatitis consistently in field trials.

The lack of cross immunity between the diseases caused by virus A and virus B was revealed by the following observations: (1) patients who have had infectious hepatitis are susceptible to homologous serum hepatitis when inoculated with virus B, and ( ) volunteers convalescent from serum hepatitis contracted infectious hepatitis when inoculated with virus A.[5] Actually, there was suggestive evidence that troops convalescent from yellow fever vaccine jaundice (virus B) were more susceptible when exposed to the epidemic disease (virus A). Of interest in this regard is an early report of Oliphant [6] that patients recovered from serum hepatitis were immune when inoculated with a strain of virus presumed to be hepatitis virus A obtained during World War II in Italy. However, the incubation period of the disease occurring in the controls was 85 to 106 days; this is reminiscent of the behavior usually associated with virus B, suggesting strongly that the Italian strain did not fall in the category now regarded as virus A, even though it was acquired in an area where infectious hepatitis was epidemic. Two other similar examples of the recovery of hepatitis virus (presumably B) from the blood of persons living in areas where infectious hepatitis was endemic were subsequently reported from the Middle East [7] and Germany.[8] It would appear, then, that at least two viruses or two strains of virus may coexist in the same area, producing two forms of hepatitis that are clinically indistinguishable although immunologically distinct. The potential epidemiologic confusion that may arise

of infectious hepatitis and arsenotherapy hepatitis in the British Navy in 1941 194 and 1943 with the peaks of incidence of the latter occurring to 3 months after the maximum incidence of the former The institution of improved techniques for the sterilization of syringes and needles was associated with a sharp decrease in the incidence of arsenotherapy jaundice These observations were interpreted as being consistent with the hypothesis that both types of disease were caused by the same icterogenic agent with the prolonged incubation period of the arsenotherapy jaundice (average 158 days) a result of the parenteral route of inoculation The importance of this as a conditioning factor in the length of the incubation period has been suggested by others[15] although the evidence for it is not impressive Actually in rebuttal of this concept are the results of limited experiments in volunteers with one strain of virus A that produced hepatitis without significant difference in the length of the incubation period following oral and parenteral inoculation[16] Parr[17] emphasized that individual differences in the host also appeared to alter the incubation period in men who received the same dose of virus B in single lots of contaminated yellow fever vaccine since their incubation periods fell within wide margins in a typical distribution curve In addition when a similar group of inoculated men were moved to less favorable conditions of life the incubation period was stated to be significantly shorter

All age groups are susceptible to serum hepatitis The disease has been said to be milder in children although serious outbreaks among infants with high mortality have been recorded Since it is impossible to measure the immune response following infection with virus B it is not known whether this general susceptibility is due to (1) insufficient exposure of the population to virus because of the artificial way of transmission ( ) the failure to develop adequate immunity following exposure or (3) the existence of multiple strains of virus that are immunologically unrelated The severity of the disease and the mortality in previously healthy young adults are similar to those in infectious hepatitis However when patients of any age are previously debilitated by illness or wounds the mortality increases sharply

Explosive outbreaks of serum hepatitis may occur following the inoculation of a group of persons with human blood or certain of its products contaminated with hepatitis virus B An outstanding example of this was furnished by the experience of American troops in World War II with some lots of contaminated yellow fever vaccine[18] When the distribution of cases was plotted according to the interval between vaccination and onset of jaundice there was a symmetrical distribution over a 7 month period with a unimodal curve reaching a peak during the twelfth week after vaccination The incubation periods ranged in general from 28 to 180 days

the maintenance of this infection in nature is well established and of particular interest in this regard is the evidence suggesting that hepatitis virus may be transmitted by infected mothers to infants *in utero* [10]

Serum hepatitis undoubtedly occurs wherever human blood or certain of its products are used or where parenteral penetrations are made It has been described in such widely separated areas as the United States Russia England Sweden and the Middle East [11] The increasing frequency of parenteral inoculations may in some measure account for the increase in the incidence of hepatitis during the past decade

Since the occurrence of serum hepatitis is dependent according to present definition on artificial inoculation its *seasonal* incidence is probably closely related to the time of preceding inoculation and the concentration of available virus The possible impact of artificial transmission of virus on the seasonal incidence is illustrated by the experience of American troops with hepatitis in Germany from 1944 to the present [1] During this period the morbidity ranged from four to eighteen per thousand per annum In the winter of 1944–1945 the incidence of disease rose sharply to epidemic proportions and declined in the spring Thereafter although somewhat higher morbidity occurred in the winter the seasonal variation was far less evident This and the continued relatively high incidence of disease constitute an endemiologic pattern that is difficult to explain although speculations have been made on the roles of increased fraternization and/or artificial transmission of virus in maintaining the high morbidity A study of the duration of residence in Germany before the onset of hepatitis showed that although it was uncommon for American soldiers to contract hepatitis before 4 to 5 months of residence in Germany the morbidity reached a peak at the end of one year and thereafter declined so slowly that it was not until 30 months after arrival that the rate could be said to be low It is unknown whether the prolonged initial delay of American occupation troops in acquiring hepatitis was the result of delay in exposure of the long incubation period of artificially transmitted virus B or a combination of both All of these concepts are tenable and in regard to the second it is of interest that Evans [8] recovered virus from the blood of a soldier with hepatitis in Germany that produced the disease in volunteers after a long incubation period

The known coexistence of virus A and virus B in the same areas suggests the possibility that the relationship between them is under certain circumstances closer than is commonly suspected Attempts have been made to relate outbreaks of serum hepatitis to preceding epidemics of infectious hepatitis Ruge [13] described the maximum incidence of hepatitis in men in the German Navy receiving antiluetic therapy as being in January some two months after the autumnal peak of infectious hepatitis More recently Ellis [14] described an apparent relationship between the seasonal incidence

of infectious hepatitis and arsenotherapy hepatitis in the British Navy in 1941, 1942, and 1943, with the peaks of incidence of the latter occurring 2 to 3 months after the maximum incidence of the former. The institution of improved techniques for the sterilization of syringes and needles was associated with a sharp decrease in the incidence of arsenotherapy jaundice. These observations were interpreted as being consistent with the hypothesis that both types of disease were caused by the same icterogenic agent, with the prolonged incubation period of the arsenotherapy jaundice (average 158 days) a result of the parenteral route of inoculation. The importance of this as a conditioning factor in the length of the incubation period has been suggested by others,[15] although the evidence for it is not impressive. Actually, in rebuttal of this concept are the results of limited experiments in volunteers with one strain of virus A that produced hepatitis without significant difference in the length of the incubation period following oral and parenteral inoculation.[16] Parr[17] emphasized that individual differences in the host also appeared to alter the incubation *period in men who received the same dose of virus B in single lots of* contaminated yellow fever vaccine, since their incubation periods fell within wide margins in a typical distribution curve. In addition, when a similar group of inoculated men were moved to less favorable conditions of life, the incubation period was stated to be significantly shorter.

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Explosive outbreaks of serum hepatitis may occur following the inoculation of a group of persons with human blood or certain of its products contaminated with hepatitis virus B. An outstanding example of this was furnished by the experience of American troops in World War II with some lots of contaminated yellow fever vaccine.[18] When the distribution of cases was plotted according to the interval between vaccination and onset of jaundice, there was a symmetrical distribution over a 7 month period with a unimodal curve reaching a peak during the twelfth week after vaccination. The incubation periods ranged, in general, from 8 to 180 days.

Of particular importance during the past few years has been the recognition of outbreaks in clinics where incompletely sterilized instruments were used and where the chances of contamination by carriers of virus were frequent. Characteristically these cases appeared in a straggling manner throughout the year apparently dependent in part on the number of available carriers and the degree of cleansing and sterilization of equipment. It has also been suggested that the pattern of such outbreaks may be influenced by the fact that virus A may be transmitted parenterally as well as virus B. Wewalka[19] described two interesting groups of cases in a clinic for antiluetic therapy (Figures and 3). In 1947 an outbreak of hepatitis occurred with a preponderance of cases 16 to o weeks following the beginning of therapy. In 1948 a second outbreak occurred however with two peaks of incidence — one appearing 4 to 8 weeks after the institution of therapy and the second peak appearing 16 to o weeks after the start of therapy. Of particular interest was the fact that certain

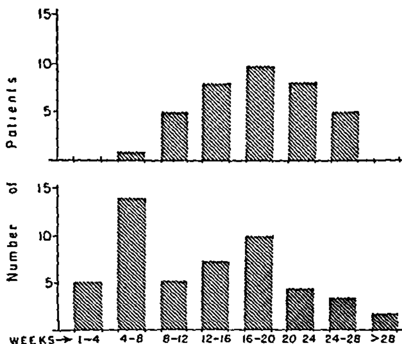


FIGURE 2. Number of cases of hepatitis occurring in varying incubation periods in a clinic for antiluetic therapy. The incubation periods are marked (in weeks) from the first injection to the onset of jaundice. The upper diagram represents the situation from December 947 to February 1948. The lower diagram represents the situation from September to November 1948. (From data given in a chart by Wewalka F. Zur Epidemiologie des Ikterus bei der antisyphilitischen Behandlung.[19])

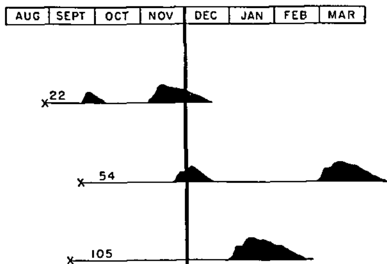


FIGURE 3 Cases of hepatitis occurring in a clinic for antiluetic therapy The numbers refer to the incubation periods of the first attack (From data given in a chart by Wewalka F Zur Epidemiologie des Ikterus bei der antisyphiliti chen Behandlung [19])

patients had the onset of hepatitis after a short (days) or moderately prolonged (54 days) incubation period followed by recovery and subsequently by a second attack after a long incubation period This evidence strongly suggested again the coexistence of at least two immunologically distinct icterogenic agents that in this situation had been transmitted parenterally possibly simultaneously Of note was the fact that the outbreak of serum hepatitis had preceded the outbreak of short incubation period disease That this should occur is indeed against the concept that the long incubation period of serum hepatitis may be explained simply as a result of parenteral inoculation of virus A

It is evident from all this controversial data that the epidemiology of serum hepatitis is closely associated with that of the epidemic disease The unraveling of the poorly defined ties that bind them together awaits the development of specific serologic methods

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14

Epidemiology of Hepatitis — Military Experience

ROSS L GAULD MD

(Washington DC)

Historically hepatitis has been outstanding as a military disease It has been a feature of all major wars for the last century and a half with a tendency for its incidence to recede with the advent of peace The experience of the United States Army in recent years has followed this general pattern However hepatitis is still prevalent in the army and although we were not at war in 1955 admissions for hepatitis to United States Army hospitals were at a rate of slightly over 2 per 1000 strength for the year [1] At this time the army was deployed in many countries and there was considerable variation in the rate in different areas In Korea where the troops lived under field conditions the attack rate was eight times that current in the United States and the occupation troops in Europe had twice the rate which prevailed in the army in the United States

It is of some interest to note that during 1955 only per cent of the hospital admissions for hepatitis were ascribed to serum hepatitis To interpret this finding we must bear in mind the fact that during 1955 the army was not engaged in combat operations Consequently blood and blood products were being used less extensively than would be the case if large numbers of wounded had to be cared for The per cent probably represents a minimum percentage for peacetime but comparative data are lacking

It is axiomatic to say that measures for the control of hepatitis must be based on the epidemiology of the disease To the epidemiologist hepatitis comprises two separate diseases each with its own distinctive characteristics [3] There is much we do not know about the epidemiology of serum hepatitis but we *do* know that the majority of cases of this disease are infected through the parenteral administration of blood and blood products [4] The control of serum hepatitis will be accomplished when we learn how to make these products safe The mechanics of the transmission of infectious hepatitis have not been as certainly determined Investigations along this line have been hampered by our failure to propagate the etio

logical agent outside the human host. Nevertheless, we have acquired certain facts as dividends from experiments using human volunteers. For instance, we know the agent is present in the blood stream during the acute disease and that it is discharged from the body in the intestinal excretions. We also know the human host can acquire infectious hepatitis *through ingestion and by parenteral inoculation* [6, 7] We are not so sure whether infection by these two routes can account for all or even a majority of the cases. This is still largely a matter of opinion.

The findings of epidemiological investigations into the natural spread of infectious hepatitis made in the army during World War II pointed to the fact that this disease is usually transmitted by some form of person-to-person spread [3, 1] Infection with the agent was shown to produce a definite resistance but not an absolute immunity, and inapparent infections were inferred on sound epidemiological grounds [7] All inferences were based on the premise that the host-parasite relationship between man and the agent reflects itself in the distribution of the naturally occurring disease.

Distribution in Military Populations In military populations infectious hepatitis generally appears as single cases, widely scattered with respect to time, place and person, and there is no traceable chain of transmission. The company is the housekeeping unit of the army, roughly comparable to the civilian household because the men work, eat and live together. Yet it is unusual for more than one or two cases to occur in a company at the same time. The peaks of incidence shown by large bodies of troops, such as armies or divisions, follow a seasonal trend and occur in the fall and winter. These peaks are the result of adding together the experience of many companies. They occur because more companies report cases at the same time and not because localized epidemics are occurring in individual companies. Indeed, in the army, localized epidemics are the exception rather than the rule.

The experience of the American Army during the first winter of the Korean War serves to illustrate the widespread distribution of infectious hepatitis in a military population. The disease was epidemic at this time and in February 1951 hospital admissions reached a peak monthly rate of 35 per 1000 strength per year. During the first 3 months of the year a total of 573 cases were evacuated from infantry units alone to the hepatitis center in Kyoto, Japan. As will be seen in Table 1, these cases were widely scattered, and 65 to 70 per cent of companies involved had only one case during any given month. Unfortunately, the total number of infantry companies committed at this time is not known, but of those companies involved there was an average of less than   cases per month per company. This widespread distribution of cases is similar to that reported in

TABLE 1

DISTRIBUTION OF 573 CASES OF INFECTIOUS HEPATITIS ADMITTED TO HOSPITAL FROM INFANTRY UNITS IN KOREA 1951 BY MONTH AND NUMBER OF UNITS INVOLVED

	January	*February*	*March*
Number of cases	144	229	200
Number of companies with cases	100	131	122
Average number of cases per company	1.4	1.8	1.6
Per cent of companies involved with only 1 case	73	64	66

1945 from the Fifth Army in Italy[6] and from the occupation army in Germany.[3]

Although there were approximately 35,000 cases of infectious hepatitis among American troops in the Mediterranean theater in World War II, common source outbreaks were rare and only two water borne epidemics were reported. Of these only one was adequately studied and this involved 86 cases in a single battalion.[6, 8] Since World War II, occasional small common source epidemics have been reported in the armed forces,[9, 10, 11] but although some such outbreaks are undetected, it is unlikely that this type of occurrence can account for more than a small fraction of the total cases. In this respect there may be a difference between civilian and military experience. If so, it is probably related to the stringent water and food discipline enforced through military regulations. This I admit is a hypothesis which has not yet acquired the dignity of a theory. Failure to account for the majority of cases of infectious hepatitis through contaminated food and water does not rule out ingestion as the method by which the infection is acquired but does seem to indicate current food and water sanitation regulations are reasonably adequate. However, these measures do not control the disease and attention is thus focused upon the hypothesis that the main route of transmission is some form of person to person contact. This premise is difficult to test, particularly because a direct chain of transmission cannot be traced from case to case. Testing demands a suitable situation and these situations are not common in shifting military populations.

Seventh Army Experience. The story of the spread of infectious hepatitis in the Seventh Army during and following its invasion of Southern France allows us to critically examine the hypothesis. The circumstances surrounding this disease in the Seventh Army were investigated at the time and a report of the findings was submitted through channels.[12] For various reasons this has not been published.

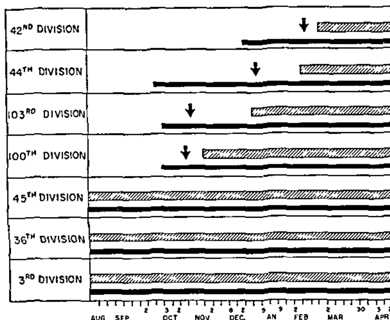
On August 15, 1944 the Seventh Army invaded Southern France. This

logical agent outside the human host. Nevertheless, we have acquired certain facts as dividends from experiments using human volunteers. For instance, we know the agent is present in the blood stream during the acute disease and that it is discharged from the body in the intestinal excretions. We also know the human host can acquire infectious hepatitis through ingestion and by parenteral inoculation.[5, 6, 7] We are not so sure whether infection by these two routes can account for all or even a majority of the cases. This is still largely a matter of opinion.

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SPREAD OF INFECTIOUS HEPATITIS IN 7TH ARMY—1944–1945

Figure 1

October to late December During this period it did not have contact with any of the infected units and remained free of hepatitis However in late December the division was placed in the line alongside the 100th Division which by this time was infected About 6 weeks later the first case appeared in the 44th Division

63rd Division Throughout the campaign most newly arrived divisions were committed as units A division is a large body of troops about 18 000 men and usually has 3 regiments of infantry In the divisions considered in Figure 1 the contact histories of the regiments were similar and allowed the division to be considered as a single entity This was not true for the 63rd Division where the regiments must be considered separately because they had different contact histories The story of this division is illustrated in Figure 2 The infantry regiments were sent overseas separately and went into battle before division headquarters and the ancillary

army had been reactivated in Italy during the early summer and was composed initially of elements transferred from the Fifth Army On D Day, its striking force consisted of three infantry divisions all of which had served for over a year in North Africa Sicily and Italy During the winter of 1943–1944 all three divisions had passed through epidemics of infectious hepatitis The disease continued to smolder in the ranks during the spring and summer and if the occurrence of clinical cases can be taken as an index all three units were thoroughly seeded with infectious hepatitis when they went to France As the army advanced northward and made contact with troops from the Normandy beachhead its size was increased from time to time by the addition of units which came directly from the United States These units were free of hepatitis when they joined the Seventh Army and thus set the stage for a study of the spread of infectious hepatitis from the infected units which had been in Italy to the noninfected units which came directly from the United States

If the hypothesis be valid that the main route of transmission of infectious hepatitis is some sort of person-to-person contact the appearance of the disease in a previously noninfected unit must always be preceded by contact with an infected unit and in addition must be independent of the time the units joined the Seventh Army Furthermore there must be a fairly close association between the time of contact and the appearance of cases These criteria require a definition of contact During the periods of active combat contact between units of the same army is quite restricted and occurs generally in one of two ways Either the unit relieves or is relieved by another unit or alternately both may fight alongside each other In this investigation contact was said to have occurred if either of these two events took place between infected and noninfected units The findings with respect to seven divisions of the Seventh Army are illustrated in Figure 1 Briefly they were

(1) During the first 14 weeks of the campaign all evacuations for infectious hepatitis were from units which had served in Italy Within these units the incidence remained low until after they had received large numbers of replacements to make up for battle losses These replacements came directly from the United States and as their proportion of the total strength increased the incidence of infectious hepatitis rose

( ) During the course of the investigation it was possible to obtain adequate records of the contact experience of 5 of the 6 infantry divisions which came to the army free of infectious hepatitis Although there was variation in the length of time elapsing between assignment to the Seventh Army and contact with an infected unit contact always preceded the appearance of infectious hepatitis by a period of between 2 and 6 weeks The 44th Division is of particular interest because it fought on the extreme left flank of the army for two and one half months from early

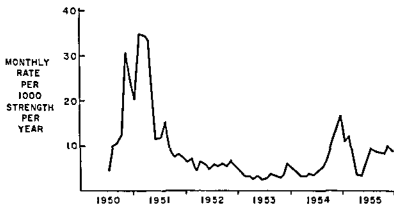
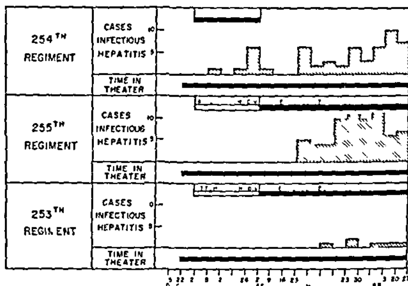


FIGURE 3

of person to person contact The findings do not in themselves constitute proof nor do they eliminate all other possible modes of transmission However if the major factor in the spread of infectious hepatitis is per sonal contact then measures designed to prevent other modes of trans mission must necessarily be of limited value This partially explains the recent experience in Korea which is illustrated in Figure 3 During the first year of the Korean War infectious hepatitis rates among American troops were high and an attempt was made to control the disease by treating all water supplies to give a residual of 5 parts per million of free chlorine This procedure was instituted in 1951 and became generally effective in April shortly after the rates had begun to fall The incidence continued to fall and reached a low level which persisted during 1952 and 1953 Many believed this demonstrated the value of adding excess chlorine to the water as an effective means of controlling infectious hepatitis However in 1954 although the excess chlorination was continued the trend was reversed and the rates again rose to the level present when the program was put into effect This was a sincere effort at control and its lack of success was probably not due to failure to prevent water borne hepatitis but because water is not the main route of transmission in the armed forces A consideration of the epidemiology of infectious hepatitis yields *no reason to expect control can be achieved in this way*

SUMMARY

Evidence has been presented to illustrate the wide scatter with respect to time place and person of the cases of infectious hepatitis which occur



INFECTIOUS HEPATITIS IN 63RD DIVISION 7TH ARMY 1945
RELATION BETWEEN ONSET OF CASES AND EXPOSURE TO INFECTED UNITS

63RD DIVISION RECONSTITUTED

FIGURE 2

troops arrived. Shortly after coming to France on December 17, they formed Task Force Harris and defended the Rhine flank. This task force was dissolved on December 30, at which time the 254th Regiment was attached to the 3rd Division, an infected unit, while the 253rd and 255th Regiments were attached to the 44th Division,* a hepatitis-free unit. The regiments remained as integral parts of these divisions for about 6 weeks, but on February 9 the 63rd Division was re-formed under its own command. As shown in Figure 2, the first case of hepatitis in the 254th Regiment appeared 2 weeks after it was attached to the infected 3rd Division and cases of the disease continued to occur in this unit throughout the rest of the campaign. On the other hand, not a single case was evacuated from the 253rd and 255th Regiments while they were with the 44th Division. Cases began to appear only after the division had been reconstituted and the two regiments came in contact with the now infected 254th Regiment.

Korean Experience. The evidence presented is consistent with the hypothesis that in the Seventh Army infectious hepatitis spread by some form

* One battalion of the 255th Regiment was attached for a few days to the 100th Division.

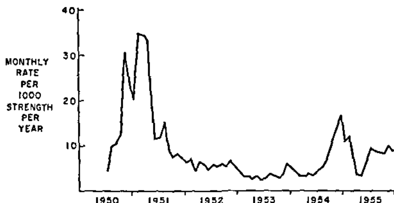


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SUMMARY

Evidence has been presented to illustrate the wide scatter with respect to time place and person of the cases of infectious hepatitis which occur

in the military forces Additional support is given to the hypothesis that the main method by which this disease is transmitted is some sort of person to person transfer Attention is drawn to the minor role played by food and water as disseminating agents in the armed forces Diseases spread by personal contact are notoriously difficult to control particularly if the situation is complicated by inapparent infections Infectious hepatitis should be recognized as a disease of this type Restrictive measures are impractical as a means of control and no vaccine is available Thus at the present time we are reduced to measures which are directed against secondary modes of spread Too much should not be expected from such procedures

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15

Certain Epidemiological Features of Infectious Hepatitis During the Delhi Epidemic, 1955–1956

R VISWANATHAN MD
(New Delhi, India)

I am very grateful to the organizers of this symposium for giving me an opportunity to contribute to the discussion on epidemiology of infectious hepatitis with reference to the large scale epidemic which occurred in Delhi India during the months of December 1955 and January 1956

Soon after the commencement of the epidemic the Indian Council of Medical Research instituted an enquiry under my direct supervision on the different aspects of the epidemic including epidemiology I was directly responsible for the epidemiological studies of the disease during the epidemic while other aspects of the inquiry were under other workers for instance Dr Smetana and Dr Gupta were in charge of histopathological studies Dr Work and Dr Melnick were in charge of virology studies while clinical studies were undertaken by some of the physicians in the hospitals

Epidemiological studies were made on the basis of the data collected on prescribed forms regarding 3786 reported cases and on the basis of sample surveys conducted in a systematic manner in order to obtain more reliable information regarding the incidence of the disease during the epidemic

All aspects of the inquiry have been completed and the results have been submitted to the Indian Council of Medical Research who intend to publish the material very shortly

For the purpose of this symposium I wish to give a short resumé of the epidemiological information that we gathered about the disease

As a result of the sample surveys the total number of cases of infectious hepatitis with frank icterus which occurred during the period of the epidemic is considered to be in the neighborhood of 29 300 in an estimated population of 1 6 million As according to Neefe over 70 per cent of the cases occurring in an epidemic are without manifest jaundice the total incidence of icteric and nonicteric cases can be estimated to be in

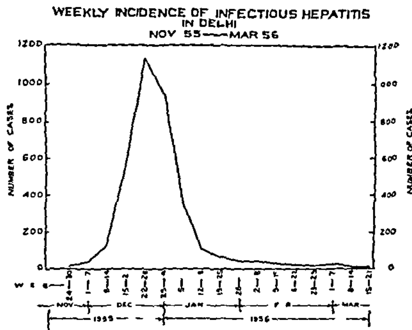


FIGURE 1

the neighborhood of 97 000 The epidemic lasted only for about 6 to 7 weeks from about December 1 1955 to about January 20 1956 The epidemic curve prepared on the basis of reported cases has a unimodal peak and its distribution is of the normal type (Figure 1) The steep rise of the curve the equally steep fall and the restriction of the duration of the epidemic to about 7 weeks are indicative of an explosive epidemic caused by a massive exposure occurring within a short period of time It is also significant that no secondary wave of the disease had been noticed in the Delhi population during subsequent weeks The incidence was widespread and occurred almost simultaneously in widely scattered areas There is definite evidence to show therefore that there was a common source and common vehicle of infection

The sample survey has shown that there is significant variation in the incidence of the disease in different regions of the city The incidence rate in that part of the city whose water supply is derived from the Municipal Water Works at Wazirabad was 2 05 per cent while the remaining areas which derive water supply from other sources had an incidence of only 0 24 per cent There is sufficient epidemiological evidence to show that the infection causing the epidemic was the result of contamination of river water before it was pumped into the waterworks at

Wazirabad The Jaundice Inquiry Committee appointed by the Delhi State Government has shown in their report that contamination of water supply at Wazirabad occurred as a result of sewage from the Najafgarh drain being pumped along with the river water at Wazirabad Pumping Station from the tenth to the sixteenth of November 1955 Epidemiological studies confirm this observation

It has been possible to estimate the incubation period for the disease during the epidemic from data collected from people who visited Delhi from outside at the time of massive contamination of municipal water supplies I obtained information from administrative medical officers of states regarding the incidence of hepatitis among this group Though over 754 cases were reported by the administrative medical officers accurate information about the period of stay and the date of onset of the disease was obtained only in respect to 61 patients On the basis of this information I have calculated the incubation period to be 18 to 62 days This corresponds to the range of incubation based on the assumption that the massive exposure of infection occurred between the tenth and sixteenth of November and because of the fact that the peak incidence of the disease in Delhi during the epidemic was between December 20 1955 and January 4 1956 It is possible that the longer incubation period for the disease noticed during this epidemic might be due to the attenuation of the virus through superchlorination and alum precipitation instituted by the authorities immediately when they came to know that the contents of the Najafgarh drain were being pumped into the water works Such measures succeeded in preventing water borne bacterial diseases like diarrhea dysentery and so forth but they were evidently not sufficiently effective against the virus of hepatitis

Surveys have shown that the incidence was highest in the age group from 15 to 39 years (2 9 per cent) somewhat lower in the age group of 40 and over (2 per cent) and still lower in the age group from birth to 14 (1 per cent)

The rates for males were higher than those for females However such differences are not considered to be significant

Incidence among pregnant women was nearly three times higher than among nonpregnant women While the general mortality rate was 0 99 per cent during the epidemic the mortality rate among pregnant women was as high as 10 per cent

Sample survey has shown that the incidence in higher income groups was significantly higher than in the lower income groups as indicated by incidence rates by type of houses While people of the higher income groups living in pucka houses and bungalows get their drinking water from municipal taps in their own houses those living in huts and mud houses have to get their drinking water from street taps Both groups no

doubt must have drunk infected water during the period of contamination. The dose of infection would necessarily have been different in the two groups as those residing in huts and mud houses had restricted water supply. In this group in certain regions, the entire incidence was confined to males aged 15 to 39. This may also be accounted for by the fact that the dose of infection was necessarily higher for people in the 15 to 39 age group as they go out to work and consume more water from public taps. A more probable reason for the difference in the incidence in the two economic groups is that those living and working under unhygienic conditions would have acquired greater resistance through previous exposures to infection.

16

*A Water-Borne Urban Epidemic of Hepatitis**

JOSEPH L MELNICK PH D
(New Haven Connecticut)

Water borne outbreaks of hepatitis have been observed in the past but none has been of the scale of the recent epidemic which occurred during last December and January in Delhi India During the epidemic period I was fortunate to have been in India working as a temporary staff member of the Rockefeller Foundation at the Virus Research Center in Poona The director of the Virus Research Center Dr Telford Work and I were invited to Delhi to participate in virological studies of the epidemic these studies were part of a series of investigations which the Indian Council of Medical Research had organized

This report should be regarded as preliminary In the near future the Indian Council of Medical Research plans to publish a series of papers on this epidemic in which the detailed investigations carried on by Indian medical scientists will be recorded so that more complete data concerning this water borne urban epidemic will be available for all students of hepatitis In addition to the information made available to me by Indian medical scientists other data were obtained during the period which I spent in India working under the auspices of the Indian Council of Medical Research and the Rockefeller Foundation The laboratory work on hepatitis at the Virus Research Center was performed chiefly by my colleagues P N Bhatt A K Thomas T Work and S Melnick It is obvious that I am merely one member of a large team of investigators who studied this epidemic and that for the most part I shall be summarizing the findings of others

The first problem which faced public health authorities was to determine the nature of the disease Although a number of people came to the hospitals and some of these went into hepatitis coma the great

* Much of the material reported was obtained while the author as a temporary staff member of the Rockefeller Foundation was attached to the Virus Research Center Poona India In preparing this invited paper the author has drawn heavily on the published Report of the committee constituted for the purpose of enquiring as to how far the pollution of Jamuna water during November 1955 was responsible for the outbreak of jaundice in Delhi in the subsequent two months"[1]

majority of cases were mild being characterized simply by "yellow eyes," that is icterus of the conjunctiva and sclera. However this was often accompanied by anorexia and general weakness. Yellow fever was hardly considered as it is transmitted by *Aedes aegypti* which is scarce in Delhi especially during December and January. Leptospiral jaundice was considered more seriously but was ruled out on the following basis. *A number of convalescent serums were obtained from patients in Delhi* and were tested in Poona against leptospiral antigens in the complement fixation (CF) test.* Leptospiral antigens as used in these tests possess broad reactivity and antibodies produced in man by one type are known to cross with CF antigens of other types. Leptospirosis did not appear to be a cause of the jaundice epidemic in Delhi, for as is shown in Table 1 the convalescent serums obtained about three weeks after hospitalization failed to react with five leptospiral CF antigens whose potency was proven in the same series of tests. This supported the view that the epidemic was one of infectious hepatitis—in agreement with the epidemiological, clinical and pathological findings made by a number of investigators.

As this is the first recorded epidemic of hepatitis in Delhi, one had to consider whether this might have been a new disease for Delhi or whether it was an epidemic of a disease already known to the area. Actually, in the past few years hepatitis had been receiving increasing attention by Indian medical scientists, and particularly the incidence of severe hepatitis among pregnant women had been under investigation in Gwalior and in Delhi. Other observations also showed that hepatitis had been endemic in Delhi for some time. Perhaps the best informataion on this situation is

TABLE 1

ANTIBODY TESTS FOR LEPTOSPIRAL ANTIBODIES IN DELHI HEPATITIS PATIENTS AND CONTROLS

	Control Serum Titer	*Control Antigen Titer*	*Convalescent Serums Hepatitis Patients in Delhi*	*Control Serums**
Leptospira hyos	1:128+	1:8	0/11 †	0/7
L. grippotyphosa	1:128+	1:4	0/12	0/7
L. sejroe	1:128	1:8	0/10	1/7
L. pomona	1:128+	1:4	1/4	1/3
L. icterohaemorrhagiae	1:128+	1:4	0/13	0/7

* Obtained from young adults in the Delhi area in the course of previous studies of the Virus Research Center.

† Numerator indicates number of positive tests; denominator indicates total number of tests carried out.

* The leptospiral antigens and control immune serums were generously made available to us by Col. C. A. Gleisner and Dr. Joseph E. Smadel of the Walter Reed Army Institute of Research in Washington.

that supplied by Dr R Viswanathan[1] (See pp 07- 10) About 1 cases of hepatitis per week were being recorded in 1955 among 00 000 insured government workers The diseases among government workers because of their insurance plan are accurately recorded By extrapolation 5000 cases per year would be expected among 1 800 000 persons in Delhi or a case rate of 3 per thousand per year During the epidemic of 1955 there appear to have been about 35 000 cases of hepatitis making a rate of 20 per thousand for the one month of the epidemic

The unimodal curve of the epidemic shown in Dr Viswanathan s paper demonstrates the sharp increase in cases and the equally sudden fall The restriction of the epidemic to only a few weeks immediately suggested to the Indian epidemiologists that there was a massive exposure for a limited period of time through a common vehicle of infection This vehicle could not have been milk or other foodstuffs because they are supplied from a variety of sources in Delhi As the peak of the curve was maintained for only a week during the latter part of December this suggested that massive exposure had occurred for about a week during the previous month The spread of disease from person to person would not tend to show such a smooth uniform rise or smooth uniform fall

An analysis of the distribution of cases in the Delhi area established a marked association between cases and city drinking water[1] The committee s report[1] indicates that the three areas of the city which were on the main city water lines had approximate attack rates ranging from 3 to 6 per thousand The rest of the city may be divided into three parts South Delhi which takes its water from the Jamuna River but which is miles downstream from the main water intake plant at Wazirabad and West Delhi and Shadra communities which take their water from wells These communities had attack rates which were only 10 to 0 per cent of those on the main water lines

It was of interest to look into another situation concerning two populations living under similar circumstances but differing only in their water supply A number of troops were stationed in the Cantonment area just outside the city of Delhi Among those on the Delhi water line the attack rate of hepatitis was 50 per 1000 Among those stationed in the same general area but separated from the main body of troops and consuming well water rather than city water the rate was only 1 per 1000 The presence of disease in this latter group might be explained by the fact that the single patient had visited that part of the camp serviced by the city water supply about 5 weeks prior to his attack It is also worth considering the incidence of cases in the troops according to rank because this indicated the type of prior immunity which existed in the community In confirmation of previous observations the rate among

the officers was twice that of the troops and four times that among the sweepers who come from lower socioeconomic groups in the general population This is particularly true for the sweepers who in addition are exposed to human excrement in the course of their daily work

The question may be asked If the cases originated from the contaminated water supply how can one explain the cases which occurred in those areas which were not serviced by the city water supply The situation in South Delhi is illustrative in this connection From information made available to the committee [1] 76 cases occurred among 106 000 people giving a case rate of 0 7 per thousand Of the total population of South Delhi it is estimated that about 5000 adult workers have employment in other parts of the city served by the main water supply line It is of importance that all 76 of the cases which occurred in South Delhi occurred in this group of daily migratory workers This gives a case rate among this group of 15 per thousand which is of the same order as that found in the city as a whole

There was one notable exception to the correlation between the geographical distribution of cases and the distribution of the city water supply One group in Delhi drank the city water but had a negligible incidence of hepatitis This group was made up of the members of the foreign embassies and their families — a group which if anything would be expected to be even more susceptible to Delhi hepatitis virus than the local population However the lack of cases in this group may be attributed to their practice of drinking only boiled water

Let us turn briefly to a consideration of the Delhi water supply which daily is drawn from the Jamuna River just northwest of the city Following the monsoons the river flooded in late October 1955 As the flood receded in November the river changed its course to the far bank so that in order to get water into the city a new channel had to be cut from the new river bed at the far bank to the water intake plant at the Wazirabad Pumping Station About 700 feet south from the water intake plant a *nalla* or creek flows into the Jamuna River The *nalla* collects raw sewage for several miles upstream before it actually spills into the river During the normal course of events the sewage from this stream enters the river and flows downstream However as diagramed in Figure 1 during the period when the flood water receded and the river changed its course the raw sewage flowed by the water intake plant and raw sewage heavily contaminated the water of the city During November and December the *nalla* contained almost pure sewage for there was no storm water in Delhi after mid October It should be pointed out that Shri R S Mehta the water engineer and his staff made heroic attempts to keep sewage from entering the water intake plant

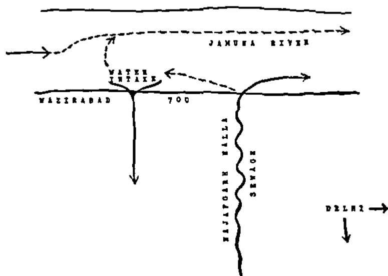


FIGURE 1 Schematic diagram of water flow in the Jamuna River in the vicinity of the Wazirabad Pumping Station Raw sewage of the Najafgarh Nalla enters the river 700 feet downstream from the water intake site of the pumping station and usually flows downstream away from the station With the change in the course of the river to the far bank at the time the flood waters receded the sewage flowed upstream and past the water intake site before it joined the river water As a result there was heavy contamination of the drinking water during the period of November 11–16

However the available resources were meager and for a period of about a week in mid November raw sewage freely entered the water pumping station

The investigating committee uncovered some pertinent information by which they could determine the extent of sewage contamination of the water supply and also perhaps the relative effectiveness of destroying enteric organisms in drinking water by means of chlorine Table 2 tabulates some of the data which appeared in the committee s report [3] During the period prior to mid November the amount of chloride present in the water supply of Delhi was about 6 parts per million On November 11th this increased to 13 and daily thereafter increased to 25 60 80 90 and 61 parts per million As the *nalla* sewage contained about 160 parts per million of chloride this would indicate that during the height of the contamination about 50 per cent of the water entering the pumping station was made up of sewage Then on the seventeenth the chloride dropped back to 6 indicating that the sewage contamination had been eliminated from the water supply

TABLE 2

DELHI WATER SUPPLY*—WAZIRABAD PUMPING STATION

Date	*Chloride (parts per million)*	*Alum (grains per gallon)*	*Chlorine Dose*	*Residual Chlorine*
November				
1–10	6	0.6		
11	13	0.8	0.5	
12	25	2.9	0.63	0.23
13	60	3.3	1.2	0.8
14	80	3.2	1.9	0.7
15	90	3.1	1.8	0.7
16	61	3.6	2.1	0.7
17	6	3.0	1.5	0.4

* Najafgarh Nalla sewage contained 160 parts per million of chloride

Recognition of contamination soon after its occurrence had been made by the staff of the water plant. They promptly increased the amount of alum for coagulation and they also increased the dose of chlorine. As shown in Table 2, the normal amount of alum was increased from 0.8 to 3.6 grains per gallon, and the chlorine from a normal dose of 0.5 to a dose of 2.1 parts per million. The residual chlorine likewise increased to 0.8 parts per million during the height of the contamination. Actually it was the high level of chlorine in the water which first informed Dr M. S. Chadha, Director of Health Services for Delhi, that there was something wrong with the water supply. The health officer was unaware of this contamination until he noticed that the drinking water had changed its flavor, for he could taste the high concentration of chlorine. This prompted him to drive over to the water intake plant, where he was able to see the extent of fecal contamination of the water entering the pumping station. When this was observed, it was already the last day of contamination, for by that time the engineering staff had been able to have new channels cut from the river to the water intake plant and the sewage was then carried downstream rather than upstream into the water pumping station. The sewage engineer had faced a most difficult problem because Delhi has no reservoir and water supplies for the city must be taken daily from the river. If he had closed down the water intake plant, then the city would have been without water. It is noteworthy that hepatitis, poliomyelitis, and typhoid have the same seasonal occurrence in Delhi, a slight peak in September and October. However, it was only hepatitis cases that showed a marked rise about 6 weeks after the sewage had entered the water supply.

The incubation period of Delhi hepatitis is worthy of attention. There

is little doubt that the heavy contamination of water from November 11th to November 16th set off the epidemic so that the incubation period of 2 to 60 days with a mode of 40 days can be established with a high degree of certainty The incubation period of 40 days is longer than that which has been noted for infectious hepatitis The investigating committee[1] suggested that this might be due to the chlorine treatment of the water for the Philadelphia group have observed that such treatment may lengthen the incubation period perhaps by partially damaging the virus A second possibility is that there are strain differences among infectious hepatitis viruses and that different strains produce diseases of different incubation periods For example Ward and Krugman[3] have recently worked with a strain of hepatitis virus in this country which produces a mild disease after an incubation period of about 40 days A third possibility is that the incubation period was lengthened because the disease was produced in partially immunized persons That the population of Delhi was partially immune is known from the occurrence of cases in the community before the epidemic and from the differences in rates among the troops and their officers presumably depending upon the extent of their prior exposure to the virus Immunity is probably relative to the challenge dose and may be overridden by exposure to a massive dose of virus However in such individuals the onset of disease may be delayed and the disease may be mild There were 73 deaths known among the 35 000 cases or a mortality index of 1 per thousand similar to that in other outbreaks It has been said that this is no greater hazard of death than is usual in Delhi

Of all the enteric infections only hepatitis occurred in epidemic form in the community There were no known increases in cases of poliomyelitis or of bacterial or amebic enteritis Those studying viral infections will be interested to know that at the time of the outbreak there did not seem to be a marked prevalence of enteric or respiratory viruses in the community While this would be difficult or impossible to analyze from statistics of recorded cases laboratory tests might be expected to reveal more information on the presence of viruses in the throats or intestines of those studied

An intensive effort was made at the Virus Research Center in Poona to isolate an agent by modern tissue culture methods* As shown in Table 3 a variety of cells were employed with the exception of monkey kidney all came from human sources Other than amnion cells the human cell lines were imported by air from the United States The number of specimens collected and tested came to 158 these consisted of stools throat swabs acute phase serums and liver obtained by biopsy

* The work at the Virus Research Center was carried out in association with P N Bhatt A K Th[illegible] I W[illegible]rk and S Melnick

TABLE 3

TESTS FOR HEPATITIS VIRUS IN TISSUE CULTURE
NUMBER OF SPECIMENS PROCESSED IN DIFFERENT CELL LINES

Specimens	*Number Collected*	*Number Tested*	*Monkey Kidney*	*Human Amnion*	*Human HeLa*	*Human KB*	*Human Detroit 6*	*Human Hep-2*	*Human Intestine*	*Human Fibroblast*
Stools	39	37	35	33	37	3	36	0	18	29
Throat swabs	49	49	39	30	49	44	13	0	32	0
Acute serums	67	67	0	30	31	48	66	31	30	0
Liver biopsy	5	5	0	0	5	0	5	0	0	0
Total	160	158	74	95	122	129	120	31	80	29
Passages attempted		71	5	2	26	79	5	0	5	0

In the course of testing 71 fluids from tissue cultures in which suspicious changes were noted were passed but no agent was isolated Not only did we fail to obtain hepatitis virus but we also failed to isolate any other enteric virus such as polio virus Echo virus or Coxsackie virus from this material which was collected chiefly from persons in the third decade of life[4] (See pp 153–168)

Because some attention has been given in this symposium to the use of Detroit 6 cells it might be worth noting here that this cell line was grown readily in the presence of high concentrations of human serum obtained locally in Poona However when the human serum was removed and replaced with animal serum prior to inoculation of the human materials the controls invariably degenerated in the course of 5 to 7 days The suggestion has been made by McLean[5] that normal human serum in various parts of the world particularly where hepatitis is endemic might at times contain a virus Such an agent might be introduced into the culture from certain human serums used as nutrient and then become latent in the culture The culture however would be protected by the antibodies present in other lots of human serum used When the human serum was removed the virus might flourish and destroy the cultures similar to the findings with polio virus[6] and adeno virus[7] Some of the paired serums from the Delhi hepatitis cases were sent under code to Dr McLean and Dr Rightsel who have isolated cytopathic agents from 6 of 8 acute phase serums but in only one or two instances from the convalescent serums of the same 8 patients[8] Whether any of these agents might be hepatitis virus remains for future work to determine

CF antigens for a number of viruses have been prepared in tissue cultures In some cases this has been possible even when specific cytopathic changes in the tissue culture were difficult to detect For this reason a number of tissue culture fluids harvested usually between the second and third week after inoculation of the hepatitis specimen were screened as CF antigens against hepatitis convalescent serums Three pools of convalescent serums were prepared from patients at the Irwin Hospital from patients at the Lady Hardinge Hospital and from patients at the army hospital in Delhi

In an attempt to remove anticomplementary activity the antigens were heated at 56 degrees C for 45 minutes and then centrifuged at 2000 revolutions per minute and some at 15 000 revolutions per minute for 30 minutes A number of antigens — about 20 per cent — were still anticomplementary after this procedure Tests were carried out on plates using the microdrop technique with overnight fixation at 4 degrees[9] Each antigen was tested against the three serum pools at 4 dilutions of complement The complement was also titrated in the presence of each antigen and

each serum pool. As shown in Table 4, most of the antigens gave negative responses. However, 3 showed fixation of complement at all levels with all 3 serum pools at 1 to 4 dilution and they were retested with 5 pairs of acute and convalescent serums at 1 to 4 and 1 to 8 dilutions. One antigen showed strong fixation with both acute and convalescent serums in out of the 5 pairs tested. The other antigens gave weak fixations with the same serums. As none of the patients showed a rise in antibodies to the tissue culture antigens, the positive CF reactions might have been positive tests for syphilis, for there is probably enough lipid material in the tissue culture fluid to have produced such a reaction. Actually, Dr. Louis Muschel has carried out a few pilot experiments to determine whether a positive CF reaction may be obtained between HeLa cells and serums containing reagin (Wassermann antibody or antiphospholipid) and obtained evidence for such a reaction. We are not attempting to propose the use of human cells grown in tissue culture as Wassermann antigen but simply wish to point out that positives of this kind occur and might be confusing in exploratory work of the nature carried out in the present experiment.

I would now like to turn to a consideration of the age group involved in the Delhi epidemic. A large majority of cases occurred in the age group between 0 and 40 years. The explanations that might be offered would include the fact that severe hepatitis with jaundice is not often seen in

TABLE 4

TESTS OF TISSUE CULTURE FLUIDS OF DIFFERENT CELL LINES FOR COMPLEMENT-FIXING ANTIGENS

Material Inoculated into Tissue Culture	*Monkey Kidney Cells**	*Amnion**	*KB†*	*Detroit 6†*	*HeLa†*	*Intestine†*	*Hep-2†*	*Total*
Stools	19	18	14	9	4	—	—	64
Throat swabs	15	15	4	13	2	8	—	57
Acute phase serums	—	—	—	18	17	3	6	44
None	1	1	2	—	1	—	—	5
Total	35	34	20	40	24	11	6	170
Anticomplementary antigens	8	6	5	8	5	1	2	35
Satisfactory tests	27	28	15	32	19	10	4	135

* Tissue culture maintenance fluid: M medium plus per cent calf serum.
† Tissue culture maintenance fluid: Eagle medium plus per cent calf serum.

the young and that the distribution in the epidemic is merely a reflection of hepatitis as it occurred in the community Secondary cases would not be expected because few persons would have remained susceptible after the widespread and massive exposure However it should be recalled that there were no observed secondary cases even in areas which were not on the water lines from the Wazirabad Pumping Station Moreover jaundice epidemics have been recorded which included a substantial number of cases in the ages between 8 and 15 years a group not prominently involved in the Delhi epidemic

A more attractive hypothesis is that the epidemic was imposed on a partially immunized population — one sufficiently immunized by the endemic prevalence of the disease to prevent major outbreaks from infection that occurs in response to normal exposure Thus hepatitis in this epidemic would have occurred only in those in whom the massive exposure was such that it overrode the immunity present in the individual Wide spread immunity in the population would have prevented secondary cases occurring at a rate above the normal one at which cases occur in Delhi This explanation fits the observations in an area such as South Delhi in which like the other areas of the city there were no secondary cases The people in South Delhi as mentioned earlier were not generally exposed to the water of the city and cases occurred only among those daily migratory workers who worked in parts of the city where they drank the Delhi water and then returned at night to their homes[1] No secondary cases occurred in South Delhi indicating that its inhabitants had sufficient immunity to protect them against normal exposure to infected persons The long incubation period of 40 days might also be explained by a delay in appearance of symptoms in persons possessing partial immunity

In addition to knowing that hepatitis was endemic in Delhi from the fact that about 100 cases per week are normally reported the relationship of station in the army to incidence of cases is such as to indicate that there must have been immunity in Delhi particularly among the poorer classes for we find that the sweepers and troops had a lower incidence than the officers who come from higher stations in life

The question might be raised Why should cases occur at all in such a widely immunized population Some data on antibody levels in populations exposed to another enteric virus that of poliomyelitis might be used as a model It has recently been shown from studies in serological epidemiology that poliomyelitis neutralizing antibodies once gained are not maintained at a uniform level in the community but actually fall with the course of time following the original exposure[9] Thus the top half of Figure demonstrates the levels of antibodies of those in the lower socioeconomic group of Charleston West Virginia — a group which

each serum pool As shown in Table 4 most of the antigens gave negative responses However 3 showed fixation of complement at all levels with all 3 serum pools at 1 to 4 dilution and they were retested with 5 pairs of acute and convalescent serums at 1 to 4 and 1 to 8 dilutions One antigen showed strong fixation with both acute and convalescent serums in out of the 5 pairs tested The other antigens gave weak fixations with the same serums As none of the patients showed a rise in antibodies to the tissue culture antigens the positive CF reactions might have been positive tests for syphilis for there is probably enough lipid material in the tissue culture fluid to have produced such a reaction Actually Dr Louis Muschel has carried out a few pilot experiments to determine whether a positive CF reaction may be obtained between HeLa cells and serums containing reagin (Wassermann antibody or antiphospholipid) and obtained evidence for such a reaction We are not attempting to propose the use of human cells grown in tissue culture as Wassermann antigen but simply wish to point out that positives of this kind occur and might be confusing in exploratory work of the nature carried out in the present experiment

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Throat swabs	15	15	4	13	2	8	—	57
Acute phase serums	—	—	—	18	17	3	6	44
None	1	1	2	—	1	—	—	5
Total	35	34	20	40	24	11	6	170
Anticomplementary antigens	8	6	5	8	5	1	2	35
Satisfactory tests	27	28	15	32	19	10	4	135

* Tissue culture maintenance fluid M medium plus per cent calf serum
† Tissue culture maintenance fluid Eagle medium plus per cent calf serum

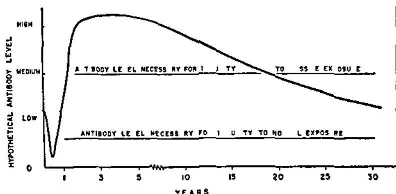


FIGURE 3 Theoretical explanation of age distribution of cases in the Delhi hepatitis epidemic Probable early acquisition of antibody and its fall over a period of years is shown A low level of antibodies suffices for protection against the usual dose of virus to which a person is exposed but a high level is postulated for protection against massive doses of virus such as occurred in Delhi in November 1955 High antibody levels would be expected in those most recently infected namely the young and therefore cases might be expected to occur with much higher frequency in those over the age of 15–20 years

to further exposure to usual doses antibodies would fall steadily with the course of time A low level of antibody seem sufficient for immunity to normal exposures in view of the fact that gamma globulin protects against the dosages involved in normal exposures (If gamma globulin is inoculated into people at much higher dosages than those used for hepatitis prophylaxis poliomyelitis antibodies which can be accurately measured are present at such low concentrations in the recipient that they can just barely be detected) Immune phenomena not being all or none we would expect that higher antibody levels would be necessary for immunity to massive exposures of the type which took place in Delhi and the cut off point for protection would be correspondingly raised If this concept is true then we would expect to find cases occurring chiefly after the age of 15 or 20 years which agrees with the observations in the Delhi epidemic.

SUMMARY

During the past December and January an explosive outbreak occurred producing clinical hepatitis in about 2 per cent of a population approximating 2 million The geographic distribution of cases together with the explosive nature of the outbreak indicated to Indian epidemiologists[1] that the infectious agent was disseminated in the population by means of the water supply The contamination of the water supply by

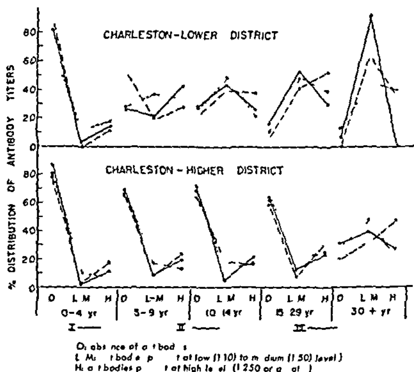


FIGURE 2 Patterns taken from studies on the serological epidemiology of poliomyelitis[9] Acquisition of antibodies in high titer is followed by their gradual decline over the years

might resemble the general population of Delhi It can be seen that these antibodies are acquired early in life that at the time of acquisition they appear at high titer and then as the years pass antibodies — although still widely prevalent in the population — are found only at low or medium levels In the upper socioeconomic group by contrast one finds that there is less exposure in the young but by the time adulthood is reached most have become infected Because adults have been infected more recently the levels of antibodies in the older populations in the upper socioeconomic groups in this country may be higher than those found in the lower socioeconomic group

Now if this situation can be used as a model for another enteric virus infection namely hepatitis we should be able to draw a hypothetical curve as that in Figure 3 for antibody levels in an area like Delhi where hepatitis is endemic One might expect that under conditions of life in Delhi young children would develop antibodies to high titers and that these would be maintained for a period of some years perhaps by multiple exposures and infections then because solid immunity would be present

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sewage was actually found to have taken place over a period of one week and occurred about six weeks before the peak of the epidemic During the period of contamination the water was treated with high doses of chlorine which might well have prevented enteric diseases, other than hepatitis from breaking out in the city It is my feeling that the outbreak can best be explained as due to an overwhelming of the immunity which already existed in the Delhi population by the extremely massive exposure to the virus

Tissue culture studies carried out with a number of cell lines including Detroit 6 failed to result in the isolation of an agent from specimens of blood throat swabs and feces The studies are noteworthy not only because of hepatitis but also because they failed to reveal any other virus in the patients studied perhaps because the viral flora in India is mainly limited to young children

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The author is also indebted to Dr D V Viswanathan Director of Public Health Poona and Lt Col S L Kalra Armed Forces Medical College Poona for a number of helpful discussions

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17

*Endemic Viral Hepatitis in an Institution: Epidemiology and Control**

ROBERT WARD, M.D., SAUL KRUGMAN, M.D., JOAN GILES, and MILTON A. JACOBS

(New York, New York, and Staten Island, New York)

Since 1953 more than 250 cases of hepatitis with jaundice have been recognized in patients at Willowbrook. In addition 65 cases occurred among attendants. During 1955 alone there were 106 cases in patients — an attack rate of 25 per 1000 — and 23 cases among attendants, an attack rate of 40 per 1000. The disease was not severe even in adults. There were no deaths attributable to hepatitis, but its protracted debilitating nature did entail considerable loss of work on the part of hospital attendants.

The Institution. Willowbrook State School is an institution for the care of mental defectives. In March 1953 there were about 3200 patients. In June 1956 the census was 4200. The rate of admission of new patients to the institution averages 15 to 20 per week.

The patients are distributed in 18 separate buildings, with roughly 100 to 250 patients per building. Meals are served to some in 2 buildings in each of which there is a central kitchen supplying 5 dining rooms. Others eat in the building where they live. No cases of jaundice have been recognized in food handlers. The water supply is the same as that for New York City. Sewage is processed in a modern disposal plant with coarse screening, settling tanks, and heat digestion with average temperatures of 87 to 90 degrees F. The effluent flows eventually into New York harbor. The sludge is dried and then may occasionally be used as fertilizer on the grounds. No vegetables are grown on the premises.

Shigella organisms are grown frequently from the feces of cases and carriers.

* The studies herein reported were conducted under the sponsorship of the Commission on Viral Infections of the Armed Forces Epidemiological Board, and supported by the Office of the Surgeon General, Department of the Army.

AGE DISTRIBUTION OF INMATES AND CASES OF JAUNDICE 1953 55

AGE (years)	INMATES No	INMATES %		JAUNDICE No	JAUNDICE %	RATE/1000
0 4	535	12 8		16	8 6	30
5 9	1017	24 3	71 6	42	22 6	41
10 14	858	20 6		34	18 2	40
15 19	576	13 9		47	25 3	81
20 29	621	14 8		22	11 8	35
30 39	313	7 5		13	7 0	40
40 49	161	3 8		7	3 8	43
50+	96	2 3		5	2 7	52
	4177	100 0		186	100 0	

TABLE 2

HEPATITIS AT WILLOWBROOK STATE SCHOOL

DISTRIBUTION OF CASES BY BUILDINGS

BUILDING NO	1953	1954	1955	1956*	TOTAL
2	3	6	9	10	28
5	4	6	19	5	34
6	1	4	8	2	15
7	0	3	4	4	11
8	1	5	7	3	16
9	0	3	10	4	17
11	0	0	4	3	7
13	0	1	2	0	3
15	0	2	4	9	15
21	6	5	5	6	22
23	7	6	4	3	20
25	1	0	19	5	25
27	1	2	2	4	9
29	0	0	1	5	6
32	9	4	6	2	21
22	0	0	0	3	3
19	0	0	0	0	0
20	0	0	0	0	0
	33	47	106**	70**	256**

** Building unknown 2 patients * incomplete

TABLE 3

Distribution of Hepatitis

1 *By season* The monthly distribution of cases over a 3½ year period is shown in Table 1 Cases occurred in every month with perhaps a slight concentration during the warm months of July through October

HEPATITIS AT WILLOWBROOK STATE SCHOOL

SEASONAL DISTRIBUTION OF CASES

	1953	1954	1955	1956	TOTAL	
POPULATION (AVG)	3500	3900	4300	4200		
JANUARY		2	8	5	15	
FEBRUARY		2	4	9	15	
MARCH		4	15	6	25	
APRIL	6	1	8	5	20	
MAY	4	3	7	8	22	
JUNE	4	4	6	7	21	
JULY	4	1	21	16	42	43%
AUGUST	9	3	7	8	27	
SEPTEMBER	2	5	7	4	18	
OCTOBER	1	10	10	2*	23	
NOVEMBER	2	6	6		14	
DECEMBER	1	6	7		14	
TOTAL	33	47	106	70	256	

* ncomplete

TABLE 1

2 *By age* The distribution of 186 cases of hepatitis according to age is shown in Table [illegible] It is compared with the age distribution of the population Over 70 per cent of the inmates are below the age of [illegible]0 years and it is in this group that 75 per cent of the cases of overt jaundice occurred The rates per 1000 over a 3 year period varied from 30 in those under 5 years to 81 in those 15 to 19 Doubtless there were many unrecognized examples of hepatitis without jaundice

3 *By building* During the last 3½ years hepatitis has been detected in 16 buildings and not in [illegible] (Table 3) Buildings 5 and [illegible]5 where the greatest number of cases were seen housed children from 3 to 15 years old The two buildings where no cases were observed provided care for tuberculous patients (#19) and psychotic elderly women who were disturbed and confined (#[illegible]0)

4 *By interval between entering Willowbrook and onset of hepatitis* It was important to know how soon after being admitted to the institution

weeks no cases of hepatitis with jaundice occurred in the groups receiving gamma globulin. In the control groups cases continued to appear for several months. Dr. Stokes offered the attractive hypothesis of naturally acquired *active* immunity superimposed on passive immunity to explain these results.

(2) Could passive-active immunity be experimentally attempted in human subjects who would later be tested for protection?

At present it is possible to present only fragmentary answers to these questions.

RESULTS

(1) *Effect of gamma globulin on endemic hepatitis.* The march of hepatitis during 1955 and 1956 up to October is shown in Figure 1. During 1955 a total of 106 cases occurred with slight concentrations in the spring and summer months. During the first half of 1956, 38 cases were fairly evenly distributed as to month of onset. On June 5th and 6th 1956 gamma globulin* was given intramuscularly to about one third of the patients in each building.† The groups to be inoculated were selected

HEPATITIS AT WILLOWBROOK

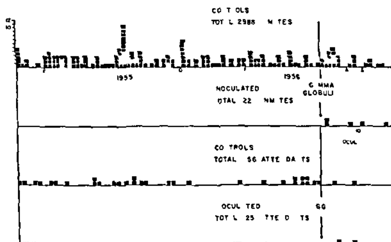


FIG. 1

* Gamma globulin was prepared by Cutter Laboratories, Los Angeles, Lot #5009 and kindly made available by the New York State Department of Health. The dose was 0.0[illegible] cc per pound of body weight.

† Except for Building [illegible] which includes isolation wards for infectious diseases including hepatitis.

INTERVAL BETWEEN ADMISSION TO WILLOWBROOK AND ONSET OF HEPATITIS

	INTERVAL	1953	1954	1955	TOTAL	%
DAYS	45 days	1	0	1	2	1
	46 99	5	3	13	21	11
	100 179	1	3	16	20	11
MONTHS	6 13 months	15	23	37	75	40
	14 24	6	4	14	24	13
	25 47	4	10	11	25	13
	48 72+	1	4	14	19	10
		33	47	106	186	99

Table 4

the patients came down with hepatitis Were they incubating the disease on entry? Could the hepatitis — either infectious or serum hepatitis — be related to injections or to some other procedure carried out on entry? The relation of length of stay at Willowbrook and onset of hepatitis is shown in Table 4 Only patients developed hepatitis within 45 days of admission Since this has been considered as the upper limit of the incubation period of infectious hepatitis it seemed unlikely that IH was either being incubated on admission or was related to injections or other procedures done at this time The onset of about one fifth of the cases was between 46 days and 6 months after entry It was conceivable that these represented examples of SH acquired on admission This appeared unlikely because no blood or blood products were given and furthermore needles and syringes are routinely autoclaved The fact that the onset of three fourths of the cases occurred 6 months or more after admission suggests that the hepatitis was acquired at the institution and was most likely infectious hepatitis

Plan of Study

In attempting to investigate and possibly control hepatitis at this institution two related questions were asked

(1) Would gamma globulin stop hepatitis in the inoculated group in the same manner as reported by Stokes and his associates working in a similar institution?[1] These workers reported that after the first 2 or 3

EXPERIMENTAL HEPATITIS

1st WILLOWBROOK STOOL POOL TITRATION BY MOUTH

GROUP	VOLUME (cc)	DILUTION	STOOL (gm)	RESULT DAY OF JAUNDICE	NO JAUNDICE / NO FED
3	20	1:5	4	42 42 44 46 47 54 54,54 58,58 62,67	1/13
2	10	1:10	1	39,39 43 49 61 0 0 0 0 0 0	5/11
1	1	1:10	0.1	39,0 0 0,0 0 0 0	1/8
1	1	1:1000	0.001	0 0 0,0 0,0,0 0	0/8
1	1	1:100,000	0.00001	0 0 0,0 0 0 0,0	0/8

TABLE 5

artificially in small groups of isolated patients. Accordingly a titration of virus in stool was carried out as follows. A 20 per cent aqueous suspension was prepared from stools of 6 patients in the first 8 days of observed jaundice. Three centrifugations were carried out: 1 at 2000 revolutions per minute and 2 at 8500 revolutions per minute for 1 hour. The supernate was heated at 56° C. for half an hour and treated with penicillin 1000 units and chloramphenicol 100 micrograms per milliliter before sterility was achieved. This sterile suspension was inoculated into 5 monkeys (1 cc. intracerebrally), 47 suckling mice, Hela and monkey kidney tissue cultures. No changes occurred in any of these test objects, and the monkeys' spinal cords and brain stems showed no evidence of poliomyelitis lesions.

The suspension was therefore deemed safe to feed. Permission was obtained to do so in groups of newly admitted children 3 to 10 years of age. They were admitted directly to and kept in separate isolation wards of the same building. They were cared for by attendants who had nothing to do with patients in the rest of the institution.

In the first trial, shown on the bottom three rows of Table 5, we started gingerly with very dilute suspensions, 10^{-5}, 10^{-3} and 10^{-1}. One of 8 children receiving 1 cc. of 10 per cent suspension in chocolate milk (0.1 Gm. of stool) came down with typical hepatitis with jaundice on the 39th day. None of the others exhibited anything suspicious during the period of observation. In the light of present knowledge of the incubation period of this strain the period of observation may not have been long enough nor were they studied as intensively as was the last group (group 3, Table 5).

In the second trial, 10 cc. of 10 per cent suspension or 1 Gm. was fed to 11 subjects. Four developed typical jaundice with confirmatory liver function tests at 39, 39, 43 and 49 days after feeding. A fifth patient

as follows. Lists were prepared of patients in each building according to duration of stay at Willowbrook. Patients with a previous history of hepatitis were eliminated. In each building every third patient on the list was given gamma globulin. Inoculated and control groups were comparable as to age, sex, race, and exposure to hepatitis at Willowbrook. In addition, gamma globulin in the same dosage was offered to attendants on an elective basis. There were 1 5 attendants who chose to receive it while 456 did not.

The subsequent occurrence of hepatitis in these 4 groups is seen in Figure 1. In the 17 weeks that have elapsed, 26 cases occurred in the uninoculated control group which totaled almost 3000. Five cases occurred in a group of about 1 00 given gamma globulin. The attack rates per 1000 for this 17-week period were 8.7 in the controls and 4 in those inoculated. A test of significance by the chi square method suggests that this difference could have occurred by chance alone 15 times in 100. Even when the cases occurring in the first 2 weeks after gamma globulin are eliminated from the calculation (4 from the controls and 2 from those inoculated), P = .06. However, it is quite possible that the absence of cases occurring in the gamma globulin group for a 5-week period (from the third through the seventh week after injection) was somehow associated with protection, perhaps passive alone, conferred by gamma globulin. During this period 1 cases occurred in the control group. There is little evidence at present of so-called "passive-active" immunity in the group which received gamma globulin. *In contrast to Stokes's experience in* which no cases of hepatitis with jaundice occurred after the second week, in the Willowbrook study 3 cases have already had their onsets 8, 11 and 17 weeks respectively after injection. To explain this difference such factors as dose and quality of gamma globulin should be considered. By the same token, the outbreaks investigated by Stokes were more explosive than those that occurred at Willowbrook. The chance of being exposed to hepatitis virus at the "right" time — when protected from infection with jaundice by gamma globulin — was perhaps less at Willowbrook than in the institutions studied by Stokes. In any event, it should be stressed that this is a report of progress only. In the course of time the accumulation of new data may bring new evidence to bear.

As far as the attendants were concerned, there is nothing to suggest a protective effect from gamma globulin.

(2) The second question was concerned with attempts to induce passive-active immunity by simultaneously injecting gamma globulin and feeding virus-containing material. Before proceeding with such trials it was important first to obtain some estimate of concentration of virus to be used. The mildness of hepatitis at Willowbrook, especially in children, and its prevalence seemed to justify attempts to induce the disease

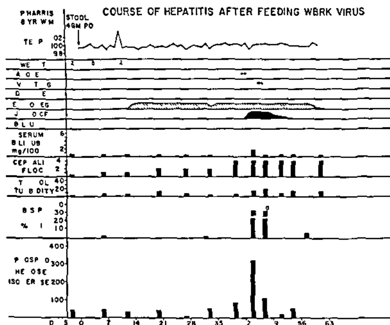


FIGURE 3

The mean normal value in adults is units with a standard deviation of 7 The upper limit of normal is considered to be 40 Extremely high values have been found in certain tissues of autopsied patients with cancer liver was over 1000-fold greater High values have been found in the serum of patients with carbon tetrachloride poisoning These represent the first determinations in children and the first in cases of hepatitis It appears that the mean average value in subjects before feeding was 31 units The median was 30 (range 6 to 54) In the patients of group 3 who developed jaundice the peak levels ranged from 68 to 1 40 The mean average was 441 The peak either coincided with the appearance of jaundice or preceded this by a few days to a week A gradual rise in the serum isomerase activity was apparent in some patients and anticipated by several days evidence of hepatitis based on clinical manifestations and other liver function tests

SUMMARY

Gamma globulin 0 01 cc per pound was given to about 1 00 inmates of a mental institution where viral hepatitis was endemic About 3000 served as controls

The attack rates during the 17 weeks following injection were 8 ,

turned up with jaundice on the 61st day. None of them had more than a very mild transitory upset associated with jaundice.

In an effort to produce jaundice in a larger proportion of the recipients 13 children were fed [illegible]0 cc. of [illegible]0 per cent suspension or 4 Gm. This resulted in hepatitis with jaundice in 12 of 13. Again the long incubation periods of 40 to 67 days were observed.

Figure [illegible] and Figure 3 show schematically the course of hepatitis in 2 of these subjects. The course illustrated in Figure 3 is an example of what was found in several subjects, namely, a prodromal illness occurring 1 to 3 weeks after feeding and characterized by transitory fever, vomiting, and occasionally diarrhea and enlargement of the liver, and equivocal liver function tests. These episodes preceded the onset of hepatitis and jaundice by 4 weeks or more. Hepatitis was confirmed by a battery of usual liver function tests.

In addition, serum phosphohexoisomerase was kindly measured by Dr. Oscar Bodansky. This glycolytic enzyme mediates the reversible conversion of glucose 6 phosphate to fructose 6 phosphate. The activity of the enzyme is expressed in units with the dimensions of cubic centimeters.

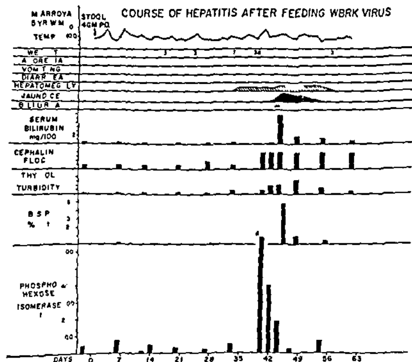


Figure 2

18

Epidemiological Aspects of Acute Hepatitis in Chile

RICARDO KATZ, M D ABRAHAM HORWITZ M D and HECTOR DUCCI M D

(Santiago Chile)

The majority of cases of hepatitis occurring in adults in Chile are isolated instances No family outbreaks or epidemics have been observed This permits us to assume that most cases are examples of inoculation hepatitis or that there exists an important number of unrecognized sub icteric or anicteric cases

Among children on the contrary small familial outbreaks of the benign form of the disease are of frequent occurrence

In order to gain information about the incidence of secondary cases we carried out the following study

Due to the lack of a specific laboratory procedure for the recognition of hepatitis serum bilirubin and flocculation tests were performed on the home contacts of adult cases of hepatitis In order to make a comparison the same laboratory tests were carried out on home contacts of patients hospitalized for other reasons a large number of apparently normal subjects and on home contacts of cases of hepatitis occurring in children

Material and Methods

The laboratory tests used were prompt direct and total bilirubin thymol flocculation cephalin cholesterol flocculation and colloidal red tests These tests were carried out in a series of 565 apparently normal subjects

Also 721 contacts of 251 adult cases of hepatitis were studied Once the diagnosis of the index case was established a home visit was made Blood was drawn from the contacts and the serums subjected to the same laboratory tests These were repeated approximately one month later in all contacts

Concomitantly 2 3 family contacts of adult patients hospitalized by diseases other than hepatitis were studied in the same way

per 1000 for controls and 4 per 1000 for those given gamma globulin The protective effect of gamma globulin was not dramatic and may have been limited to a period 3 to 7 weeks after inoculation

So far evidence of passive active immunity has not been apparent in this study

A suspension of stool from jaundiced patients in this institution has been fed in graded quantities to subjects held in isolation Mild hepatitis with jaundice was produced after long incubation periods (39 to 67 days) in 18 patients The 50 per cent infectivity (jaundiced) end point appears to be about 1 Gm of stool

The stage is set for carrying out controlled tests of passive active immunity

ACKNOWLEDGMENT

We would like to thank the various members of the Viral Infection Commission of the Armed Forces Epidemiological Board especially Dr John Paul for strong support and help in designing these trials

We are indebted to Dr Paul H Hoch Commissioner of the New York State Department of Mental Hygiene and to Dr Harold H Berman, Director of the Willowbrook State School for their splendid co operation

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Among the 721 contacts of adults with hepatitis only 4 cases of hepatitis with jaundice were observed However among 61 contacts of children with hepatitis 9 cases of icteric hepatitis and 5 cases of anicteric hepatitis occurred This difference led us to analyze the age distribution of the contacts with and without hepatitis in both groups This distribution is shown in Table 3

TABLE 3

AGE DISTRIBUTION OF THE CONTACTS OF HEPATITIS INDEX CASES AND OF THE SECONDARY CASES WHO HAD HEPATITIS

	Index Cases		*Cases of Hepatitis*	
ADULTS				
Contacts (721)				
Less than 10 years of age	114	(15.8%)	3	(2.6%)
More than 10 years of age	60	(84.2%)	1	(0.16%)
CHILDREN				
Contacts (61)				
Less than 10 years of age	50	(82%)	14	([illegible]8%)
More than 10 years of age	11	(18%)	0	(0%)

Among the contacts of adults with hepatitis 3 cases developed in children of less than 9 years of age although the contacts in this age group represented only 15.8 per cent of the total Moreover all the cases of hepatitis with jaundice among the contacts of children with hepatitis occurred in persons of less than 9 years of age

DISCUSSION AND SUMMARY

Our investigation on the familial contacts of adults with hepatitis has shown a low incidence of secondary cases This supports the impression that most cases occurring in adults in Chile correspond to examples of inoculation hepatitis

According to our results the lack of family outbreaks in relation to an adult with hepatitis is not explained by the occurrence of subclinical cases The proportion of abnormal results of the laboratory tests used in the contacts of hepatitis cases is similar to that obtained in a control group formed by home contacts of individuals hospitalized for diseases other than hepatitis

In the group of apparently normal individuals here reported a higher proportion of abnormal results of the laboratory tests than that observed by others was obtained This discrepancy is due to our lower upper normal limits prompt direct bilirubin 0.4 and total bilirubin 0.99 mg per 100 ml of serum thymol flocculation negative cephalin cholesterol

The same investigation was carried out on 61 contacts of 24 hepatitis cases younger than 14 years

Results

The percentage of positivity of the laboratory tests in the contacts of adults with hepatitis in the contacts of adult controls and in apparently normal subjects is shown in Table 1

Table 1

PERCENTAGES OF POSITIVITY OF THE LABORATORY TESTS IN 721 CONTACTS OF ADULTS WITH HEPATITIS 223 CONTACTS OF ADULT CONTROLS AND 565 APPARENTLY NORMAL SUBJECTS

	Prompt Direct Bilirubin %	*Total Bilirubin* %	*Thymol Flocculation* %	*Cephalin Cholesterol* %	*Colloidal Red* %
Hepatitis contacts (721)	11.9	3.6	21	26.3	43.1
Control contacts (225)	13.9	4.5	21	16.6	35.8
Normals (565)	16.3	4.1	11.9	21.8	20.3

The comparison of the results in the three groups shows

(1) A high percentage of abnormal tests in apparently normal subjects

(2) A slightly higher percentage of abnormal results in hepatitis contacts than in control contacts This difference is statistically significant only for the cephaline cholesterol flocculation

(3) That the comparison between contacts of hepatitis and normal subjects makes the difference erroneously significant

The incidence of hepatitis with jaundice among the contacts of adults and children with hepatitis is shown in Table 2

Table 2

INCIDENCE OF HEPATITIS IN CONTACTS OF INDEX CASES

Adults		
Index cases	251	
Contacts	721	
Hepatitis with jaundice	4	(0.55%)
Children		
Index cases	24	
Contacts	61	
Hepatitis with jaundice	9	(22.9%)
Hepatitis without jaundice	5	

DESIGNATED DISCUSSION

HANS F SMETANA M D (Delhi India) The only distinction that entitles me to discuss the epidemiology is that I know nothing about it therefore my judgment is not clouded by any special knowledge of the subject

However the student of hepatitis or of disease in general has to be interested in epidemiology and the only knowledge that I can present here is my presence in Delhi during the epidemic which, from several points of view was rather interesting

First of all it was like an experiment We know exactly the date on which the water was contaminated and how long it was contaminated We also know the supply lines of the water from which the population was affected From these data something could be calculated

However it became obvious that a large apparatus was necessary to study the epidemiology which consisted mainly of the accumulation of accurate data and in this particular situation it was extremely difficult We are very grateful to Dr Melnick for providing perhaps the most accurate information that we have during this time

The other point is this Why did this epidemic break out in such an explosive fashion It is said that there is quite a high incidence of endemic hepatitis in Delhi but perhaps the heavy infestation of the water broke through this defense mechanism of the population It also was clear that the higher bracket of Indians was more affected than the sweepers perhaps again because the latter were more accustomed to that sort of infection

GENERAL DISCUSSION

M BJØRNEBOE M D (Copenhagen Denmark) I would like to briefly illustrate one point in Dr Paul's paper by demonstrating changing age distribution of hepatitis in Denmark from 19 8 to 1956

Adult patients over 15 years of age comprise an increasing proportion of the total number of patients From 1930 to 1935 about 40 per cent of the patients were adults while since 1945 approximately 70 per cent of the patients were adults In the late 1940s we assumed this was due to the virus prevalent at that time The age distribution however has not altered since then I wonder if this has been observed in other countries

SVEN GARD M D (Stockholm Sweden) I have threatened to say something about an oyster epidemic and I think I had better do it I will be as brief as possible

In Sweden around Christmas of 19 5 an outbreak of hepatitis was found of a rather unique type The distribution of cases was concentrated

flocculation negative and colloidal red 2 If a higher upper normal limit is used the percentage of positive results in cases of hepatitis is greatly reduced

Although the group of apparently normal individuals showed a high proportion of abnormal results its comparison with the group of adult hepatitis contacts would make interpretation of the difference erroneously significant This difference disappears if the hepatitis contacts are compared with the contacts of patients hospitalized for diseases other than hepatitis

The occurrence of small outbreaks of hepatitis in children has been confirmed It is possible that the opportunities for being infected with natural hepatitis at an early age are great in Chile and that the subsequent immunity reduces the number of cases of this type of hepatitis in adults

never been in the harbor a mixture of freshly caught and stored oysters and finally some shipments consisting only of oysters stored in the harbor It was not possible afterwards to assess the different shipments in these respects but considering this it is quite remarkable that among 300 consumers who were rounded up in Stockholm, more than 50 per cent had come down with hepatitis

There were occasions when the person had entered the shop and just had a morsel not a whole oyster and still came down with hepatitis four weeks later This may serve as a justification for our attempts to try to grow hepatitis virus in oyster tissue

in three of the larger cities and scattered over 102 different towns in Sweden

The epidemic was soon traced to oysters delivered from two different wholesale establishments in a small fishing village on the west coast There were very many cases in the village around November 25th and the number of the infectious oyster meals rapidly fell off after that

This epidemic started around December 18 or 19 and ebbed away around January 15th altogether there were 6 9 cases

The rather unusual feature as compared with the cases discussed previously today *is mainly in the age group which was from 25 to 60 years* with practically no children involved at all Furthermore there is a preponderance of male patients in this material Almost 90 per cent of the patients belonged to the higher socioeconomic group It shows again the preponderance of men over women

It was soon found that in this village one person had come down with hepatitis on November 4th He had worked in the establishment on the north pier up until November 23rd On November 4th he stayed at home in bed six days later he was taken to the hospital with a typical acute hepatitis

There are flush toilets in the village and they are connected with sewers that empty directly into the channels without any purification There are three on the west side of the harbor and one on the east side

In the establishment at the north pier there was no flush toilet but a dry toilet The receptacle was emptied into the sea outside the harbor once or twice a week

Considering the fact that surface oysters brought in by the fishermen when catches were larger than the immediate demand were stored in the harbor at the piers in wire baskets that the average period of storage in the harbor seemed to be about ten days and adding to this the average period elapsing between delivery from the wholesale dealer and the date of consumption and also the fact that the retail stores were holding the oysters for some days then from the curve of the data of consumption which unfortunately came a little too early we can actually draw the conclusion that practically all 6 9 cases could be traced to those oysters stored in the harbor on one certain date

A few days before this man came down with hepatitis and stopped working at the north establishment It makes it not improbable that the dumping of the latrine on a certain date was the beginning of the actual date of infection of the bay and the oysters then present in the harbor became infected

The attack rate among oyster consumers was surprisingly high We have to take into consideration that during the critical period three types of shipments were sent from the dealers freshly caught oysters that had

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PART III

Prevention — Blood Donors and Storage Problems

Moderator JOHN R NEEFE, M D (*St Petersburg Florida*)

19

Nutrition as a Protective and Therapeutic Factor in Hepatitis

PAUL GYORGY MD
(Philadelphia Pennsylvania)

Viral hepatitis is infection centered mainly in the hepatic parenchyma and as such equivalent to a toxic damage of hepatic tissue As in any infection resistance or its reverse — susceptibility to it — depends on the interplay of specific immunological reactions and on unspecific factors In the presence of specific humoral antibodies or specific cellular immunity even massive infection may be easily combated and warded off Unspecific resistance may be related to humoral systems such as perhaps the properdin system or to increased tissue resistance determined by dietary and endocrine factors

From a pathological point of view necrosis and cirrhosis are the characteristic manifestations of injury to the hepatic parenchyma Fat infiltration is not specific enough and often too transient without concomitant manifestations of tissue reaction to be considered in itself as a truly pathological condition of the liver

In the past experimental hepatic injury was produced through exposure of the living animal to various hepatotoxins From these experimental studies it was concluded that in every instance in which the prolonged or repeated action of an agent has resulted in some degree of cirrhosis the acute effects have been degeneration and necrosis of hepatic cells[1]

It should be added that toxic injury of the liver parenchyma may also lead to more specific histological changes such as hydropic degeneration after chloroform intoxication[1] cholangitis and pericholangitis hyperplasia of biliary ducts[2 3] seen after administration of various carcinogens such as para dimethylaminoazobenzene (butter yellow) or various plant extracts containing pyrrolizidine alkaloids (represented by plants of the senecio crotalaria group and so forth) or vascular obstruction through edema and proliferation of the endothelial layer in the hepatic venules and veins (venous occlusive disease V O D) seen in infants with infantile cirrhosis in Jamaica[4] and almost certainly related to the consumption of

19

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bush tea containing hepatotoxic compounds probably again of the group of pyrrolizidine alkaloids.

More recently purely nutritional factors have been recognized in animal experiments as important primary determining causes of hepatic injury. Proper change in the composition of the experimental diet may bring about prevention arrest and even possible reversal of the underlying pathological process in the hepatic parenchyma. Endocrine factors and antibiotics also play a role in the development of dietary and toxic hepatic injury.

Dietary *factors determining experimental liver injury in rats* are summarized in Table 1.

TABLE I

DIETARY FACTORS IN LIVER INJURY

	Necrosis	*Cirrhosis*
Protein	Beneficial	Beneficial
Methionine	Beneficial	Beneficial
Cystine	Beneficial	Beneficial
Choline	No effect or injurious	Beneficial
Vitamin E	Beneficial	No effect
Dietary fat	No effect or injurious	Injurious
Vitamin B_1	No effect	Beneficial
Factor III	Beneficial	?

For both the acute and chronic forms of experimental dietary hepatic injury protein is beneficial. For the production of necrosis and cirrhosis of the liver a diet deficient in protein is one of the essential prerequisites. In experimental dietary necrosis of the liver the sulfur containing amino acids are the protective and active constituents of protein. Even if the requirement for the sulfur containing amino acids is not covered vitamin E and Factor III may prevent the development of experimental hepatic necrosis. Factor III is present in yeast and in various casein preparations and is apparently a vitamin E like substance but chemically different from vitamin E. The sulfur containing amino acids (cystine methionine) as well as vitamin E and the so called Factor III are mutually interchangeable *in the protection of the hepatic parenchyma from dietary massive* necrosis.

Only very scant information is available on the chemical nature of Factor III.[5] In our laboratory we have isolated a crystalline substance *from yeast which is a powerful antioxidant its activity is similar to that of* vitamin E[6,7] in all appearances and also with regard to the prevention of dietary massive hepatic necrosis.

The beneficial effect of protein specifically that of sulfur containing *amino acids and of vitamin E applies not only to dietary hepatic necrosis* but also to acute hepatic necrosis caused by many hepatotoxic agents such

as chloroform and other halogenated hydrocarbons Protein also reduces the toxicity to para dimethylaminoazobenzene and various hepatotoxic alkaloids [8]

For the chronic form of experimental hepatic injury namely cirrhosis protein and lipotropic factors are the leading protective dietary compounds The beneficial effect of protein in this regard is determined not only by its content of methionine as precursor of choline as the most effective lipotropic factor but also by the nature absolute amount and balance of other amino acids present This disturbed equilibrium of amino acids is found especially in proteins of vegetable origin

In the prevention of toxic cirrhosis protein may be effective through a similar detoxifying mechanism as in acute necrosis of the liver caused by the same toxic substances given in large acutely injurious amounts

Antibiotics are effective in both experimental necrosis and cirrhosis Their effect is not always predictable and may vary from time to time it is often also related to the type of the experimental diet used In general penicillin the tetracycline group and to a lesser degree neomycin all given by mouth were found to be the most effective antibiotics in the prevention of experimental hepatic injury The protection afforded by antibiotics[6, 8] in the production of dietary experimental hepatic necrosis has been widely considered in support of its toxic origin either through the mediation of the intestinal flora or through intermediary metabolites However neither observations on germ free animals[9, 10] nor more thorough analysis of the effect of antibiotics on experimental hepatic injury in both its forms necrosis and cirrhosis[7, 8, 11] have been in full accord with this thesis They are more in line with the assumption that antibiotics act as sparing agents through better or selective utilization of amino acids that is in last consequence they act through their effect on protein via the intestinal flora

The above considerations on the role of dietary factors and of antibiotics centered around the *prevention* of hepatic injury In general preventive and therapeutic measures are by no means necessarily interchangeable Specifically effective prophylaxis should and will prevent the development of the specific metabolic and anatomical changes Therapeutic efforts on the other hand have to deal with the arrest or even regression of already existing metabolic and anatomical changes For instance in the case of cirrhosis the progress of fibrosis has to be checked or even reversed in addition to repair of all other concurrent pathological disturbances Furthermore in cirrhosis not only the liver but often other organs as well such as the kidney and endocrine glands especially the gonads are found to be involved in the over all disease

In acute severe hepatic injury or in severe decompensation of hepatic function in the course of chronic injury (cirrhosis) administration of

bush tea containing hepatotoxic compounds probably again of the group of pyrrolizidine alkaloids.

More recently purely nutritional factors have been recognized in animal experiments as important primary determining causes of hepatic injury. Proper change in the composition of the experimental diet may bring about prevention arrest and even possible reversal of the underlying pathological process in the hepatic parenchyma. Endocrine factors and antibiotics also play a role in the development of dietary and toxic hepatic injury.

Dietary factors determining experimental liver injury in rats are summarized in Table 1.

TABLE I
DIETARY FACTORS IN LIVER INJURY

	Necrosis	*Cirrhosis*
Protein	*Beneficial*	*Beneficial*
Methionine	Beneficial	Beneficial
Cystine	Beneficial	Beneficial
Choline	*No effect or injurious*	Beneficial
Vitamin E	Beneficial	No effect
Dietary fat	No effect or injurious	Injurious
Vitamin B_1	No effect	Beneficial
Factor III	Beneficial	?

For both the acute and chronic forms of experimental dietary hepatic injury protein is beneficial. For the production of necrosis and cirrhosis of the liver a diet deficient in protein is one of the essential prerequisites. In experimental dietary necrosis of the liver the sulfur containing amino acids are the protective and active constituents of protein. Even if the requirement for the sulfur-containing amino acids is not covered vitamin E and Factor III may prevent the development of experimental hepatic necrosis. Factor III is present in yeast and in various casein preparations and is apparently a vitamin E like substance but chemically different from vitamin E. The sulfur containing amino acids (cystine methionine) as well as vitamin E and the so called Factor III are mutually interchangeable in the protection of the hepatic parenchyma from dietary massive necrosis.

Only very scant information is available on the chemical nature of Factor III. In our laboratory we have isolated a crystalline substance from yeast which is a powerful antioxidant its activity is similar to that of vitamin E [6, 7] in all appearances and also with regard to the prevention of dietary massive hepatic necrosis.

The beneficial effect of protein specifically that of sulfur containing amino acids and of vitamin E applies not only to dietary hepatic necrosis but also to acute hepatic necrosis caused by many hepatotoxic agents such

proof for direct connection between viral hepatitis and malnutrition or vice versa between resistance to hepatitis and good nutrition On the other hand it is logical to assume that low dietary intake of protein vitamin E and Factor III should reduce the resistance of hepatic parenchyma to any necrotizing toxic insult including hepatitis virus

The higher incidence of hepatic injury in males — and in particular the epidemic appearance of severe often fatal viral hepatitis in the postwar period in the Scandinavian countries afflicting chiefly women in menopause [3, 4] — is in good accord with the experimentally proven higher resistance of female animals to dietary injury or to hepatotoxic agents The fact that chronic constipation as Bjørneboe and his associates have shown [25, 26] was a statistically significant occurrence in Denmark in the postwar epidemic among women in menopause suffering from chronic hepatitis raises the possible connection of the liver with intestinal flora apart from the underlying endocrinological constellation

One is on safer grounds in relating the therapy and management of hepatitis to dietary factors In acute severe decompensation of hepatic function, such as in the early stage of viral hepatitis [27] or in the last stage of cirrhosis administration of protein protein hydrolysate amino acids ammonium salts or ammonia exchange resin may precipitate the development of hepatic coma [28, 29] In such instances reduction in intake of nitrogenous compounds and administration of antibiotics the latter through the intermediary of the intestinal flora may stave off hepatic coma or related neurological reactions (flapping tremor ataxia mental confusion) Murray and his collaborators found a protein rich diet distinctly dangerous in their volunteers inoculated with hepatitis virus and kept on that diet beginning with the first appearance of symptoms of hepatitis Mortality and various complications were observed in significantly higher number in the subjects with inoculation hepatitis receiving a protein rich diet than in the controls fed an ad libitum diet with a distinctly lower proportion of protein

Chalmers *et al* [30] in their extensive studies on hepatitis patients from the Korean War observed after examination in an army hospital in Japan found diet rich in protein superior in the management of viral hepatitis to a lower protein intake That this difference in observations between these two groups of investigators was due to the fact Murray and his group were dealing with hepatitis in its acutest truly initial stage whereas Chalmers and his group have been treating hepatitis in its later phases is a plausible but not necessarily binding conclusion The difference in the proportion of fat in the diets of patients of Murray and Chalmers (with their respective associates) may also be taken in consideration although in our opinion, this is of distinctly less consequence

In conclusion it is recommended that in the acute phase of viral hepa

protein and even of amino acids for instance methionine may be dangerous as the liver may not be able to metabolize or detoxify the metabolized products sufficiently In consequence the effect of antibiotics in acute severe hepatic injury may be the suppression of intestinal bacteria which form from protein potentially toxic breakdown products (ammonia amines etc) with which the severely deranged liver function is unable to cope

Endocrine and dietary factors are closely related to each other in the pathogenesis of experimental hepatic injury Among all steroids tested only the estrogenic hormones were found to prevent the development of fat infiltration and of cirrhosis of the liver in rats kept on a diet low in protein and lipotropic factors[1, 13] Testosterone methyltestosterone progesterone DOCA and cortisone were at least in the doses used in effective This beneficial protective effect of estrogenic hormones appears to depend upon an extrahepatic mechanism rather than upon direct hepatic action[14, 15] The extrahepatic effect might be mediated through the anterior pituitary gland

The prevention of cirrhosis by estrogenic hormones is in good accord with the greater resistance of female rats to injury caused by dietary deficiency (choline[16] cystine[17]) or by exogenous hepatotoxins (chloroform[18] carbon tetrachloride[19] phenobarbital[9]) compared with the marked susceptibility of male rats to the same noxious agents

The endocrine glands which have an effect on the liver are not limited to the female gonads Goitrogenic substances[9, 1] exert a beneficial effect in the prevention of dietary cirrhosis and necrosis of the liver in rats The greater the goitrogenic potency of a substance the more effective it proves to be with regard to the prevention of experimental dietary injury of the liver Thus the specific effect of goitrogenic substances on the liver may be related to their effect on thyroid function particularly to the decreased metabolic rate including the metabolism of protein

The foregoing experimental observations and general considerations may be brought at least to a limited extent in accord with the clinic and management of viral hepatitis and its sequelae

Prevention and therapy should be treated as different fields of medical endeavor

Viral hepatitis in its acute phase is a necrotizing toxic injury Its "take and course will depend more on the intensity of the infection than on the resistance of the hepatic parenchyma apart from the specific and unspecific humoral immunological factors of susceptibility It has been often claimed that widespread hepatic disease in underdeveloped countries with poor nutritional standards such as Indonesia and India has often been related to malnutrition However there is no definite and conclusive

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titis or in severe exacerbation of subacute chronic hepatitis and in de compensated cirrhosis the diet be kept low in protein supplemented with antibiotics

In convalescence or in the chronic phase of postviral hepatitis as in any form of cirrhosis not threatened by decompensation protein or lipotropic substances are beneficial They will support regeneration and may contribute to the prevention of further progression

Protein is certainly central in the prophylactic and therapeutic measures connected with experimental and clinical hepatic injury However in itself it does not necessarily represent the complete answer

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*Turbidity Reaction Mechanisms and Turbidity Measurements in the Study of Hepatitis**

JOHN G REINHOLD PH D
(Philadelphia Pennsylvania)

The use of the group of laboratory procedures commonly referred to as turbidity and flocculation reactions has contributed greatly to the study of viral hepatitis That viral hepatitis often occurs without jaundice or hyperbilirubinemia was largely established with the aid of these procedures Moreover they contributed to the proof that convalescence following viral hepatitis was delayed in some persons despite apparent clinical recovery Differentiation of the causes of jaundice has been aided by their use For certain purposes such as the examination of large numbers of blood donors they provide a great deal of information in return for a relatively small expenditure of time In addition they may disclose significant facts about patients suffering from viral hepatitis that may escape detection by far more elaborate procedures such as electrophoresis

On the other hand the turbidity and flocculation tests have limitations Under no circumstance are they specifically diagnostic Their mechanisms are only partially understood The techniques for their performance have not been adequately studied and standardized The values found in populations of healthy persons are not well defined In this paper I shall describe observations related to these aspects of the thymol test of Maclagan [1] and the zinc turbidity test described by Kunkel [2]

The work done by Mateer and his associates [3] in the Henry Ford Hospital on the thymol test nearly 10 years ago led us to adopt the pH of 7 55 for buffering the reagent as they recommended Subsequently the work of Neefe and others [4] provided convincing support for the use of this modification Regardless of which pH is selected it must be rigorously controlled and neglect of this is one of the common causes of erratic results The greatest source of variation in pH is the CO content of the

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suspensions formed by the reaction of serum and the thymol barbital reagent

This preparation was calibrated by sending samples to 50 different laboratories Over 40 replied and the mode of the reported values was adopted as the thymol turbidity equivalent The modal value so obtained yielded results that agreed with results found by Professor Maclagan when allowance was made for the difference between Maclagan s units and the unit introduced by Shank and Hoagland

From the beginning disagreement has existed concerning the limit between normal and pathological as measured by the thymol test Studies by Neefe Cambescia and others [11] a few years ago established 5 0 Shank Hoagland units as a limit including roughly 95 per cent of healthy controls This is well below the limit of 4 0 units fixed by Maclagan (equivalent to 8 0 Shank Hoagland units) We have since studied another group of healthy persons both in fasting condition and two to three hours after lunch The limits that include 95 and 99 per cent of this population are 4 0 and 4 8 units respectively in the fasting group and 5 0 and 6 0 in the postprandial group provided that the scattered high values are disregarded The latter therefore support the limits established by Neefe and associates and also those of Zieve and Hill [12] These limits differ appreciably in the fasting group from those in the postprandial group this finding refutes numerous statements to the contrary Failure to use the appropriate standards for fasting subjects as compared with postprandial ones will impair appreciably the usefulness of the thymol test

Any decision establishing a limit between normal and abnormal is somewhat arbitrary However an interesting observation has supported the selection of 6 5 units as this value The frequency of distribution of the thymol turbidities of 1035 blood donors (3 to 5 hours after a meal) is shown in Figure 1 It will be seen that this curve is bimodal with a minimum between 6 0 and 7 0 units and a second small maximum in the 7 0 to 8 0 unit region This small peak has recurred quite consistently when similar plots of large populations were made and is seen also in the data of Zieve and Hill [1] Obviously this segment of the population differs from the remainder and it is noteworthy that many carriers of viral hepatitis have thymol turbidities in this region the tail of the thymol turbidity distribution

Studies of similar distributions of zinc turbidity measurements have disclosed some unexpected results Several years ago we noticed that an unduly high proportion of Negro blood donors and hospital employees had elevated zinc turbidities It is well established that the Negro populations of Africa the West Indies and the southern United States have higher mean serum globulins than do the whites living in the same areas and evidence is available to suggest that gamma globulin accounts for most of the

distilled water used for dissolving the reagents It is essential that it be boiled shortly before it is poured on the thymol and barbital[5] Finally the reagent must be kept fully saturated with thymol partly because the solubility of thymol varies considerably with temperature and partly because the thymol protein complexes have large temperature coefficients, as we showed several years ago[6] Therefore precision in thymol turbidity measurement requires that the test be carried out at a fixed temperature and unless this is done large errors are introduced

Measurement of turbidity by means of visual comparisons is reasonably accurate but quite imprecise Measurements of turbidity can be made with considerable confidence with a well designed spectrophotometer turbidity measurements based on absorbency in suitable instruments over selected ranges of concentration are no less accurate than those that measure scattered light[7] However thymol turbidity measurements present special complications that may cause gross errors as Høyer and Jørgensen[8] have shown One cause of difficulty has been the lack of a satisfactory standard for thymol turbidity measurements Barium sulfate as advocated by Shank and Hoagland[9] may give excellent reproducibility in any one laboratory but for various reasons fails to give similar results in different laboratories The thymol turbidity readings reported by 45 laboratories several years ago when they were asked to measure the *turbidity of a standardized suspension of colloidal glass* varied more than tenfold When allowance was made for the use of two different units[1, 9] the highest reading was still nearly five times the lowest It appears that the variations were largely the result of the erratic behavior of the barium sulfate standards prepared in these laboratories However differences in the optical geometry of the measuring instruments used also are important and a given thymol turbidity may be read quite differently in photocolorimeters of differing design The particle size distribution of the turbid suspension being measured is the critical factor Barium sulfate varies greatly in particle size depending on conditions of preparation and therefore is not a suitable standard Several years ago Dr Charles Jones and Dr Roy Turner suggested the use of colloidal glass suspensions as turbidity standards Colloidal glass offers many advantages over barium sulfate suspensions yet the thymol turbidity equivalent of such preparations also differed considerably In a recent study[10] comparison of glass suspensions with the turbid suspensions produced by reaction of the thymol reagent with serum showed that the occurrence of two different thymol turbidity reactions in serum complicated the standardization one producing particles of larger sizes was due to the reaction of thymol with gamma globulin the other involved lipide and lipoprotein Eventually it was possible to select suitable conditions for sedimentation that yielded a colloidal glass suspension that resembled in particle size and in general behavior the

lipoprotein and other plasma proteins as well as with gamma globulin only gamma globulin ordinarily precipitates under the conditions existing in our experiments Siderophyllin the metal combining beta globulin has a strong affinity for zinc although much less so than for iron The possibility that a deficiency of metal binding ability existed in serums of Negroes was tested on the chance that such a deficiency allows more zinc ions to remain free to react with beta globulin Measurements of iron binding capacity of healthy Negroes showed that some had lowered unsaturated iron binding capacities However no consistent difference was found Moreover the quantity of zinc in the zinc barbital reagent exceeds enormously the total metal binding capacity of serum of either Negroes or whites so that saturation differences hardly offer a plausible explanation The serum iron concentrations seem to be about the same in the two races

Yet another difference in the reactivity of Negro and white serums with metals has been demonstrated in experiments in which ferrous iron solution was added to the serums buffered with barbital at pH 5 0 one hour before zinc sulfate solution The zinc turbidity decreased in both groups when this was done however the change was much larger in Negro than in white serums These experiments together with the others described suggest that important differences exist in the serum proteins of the two races — a factor that most assuredly must be taken into account in studying either

One other curious fact quite unrelated to the foregoing learned about the zinc turbidity test was an outgrowth of studies of serum preservation The zinc turbidity of serum which had been separated promptly from the clot was always lower than when tested on the following day regardless of mode of storage whether at 25 C 5 C or −20 C[16] However the zinc turbidity was unchanged if the serum remained on the clot during this time Separated serum protected from contact with air during the storage also had unchanged zinc turbidity readings Further studies established that CO participates in a reaction between zinc and gamma globulin for turbidity rises with CO content to a maximum then falls[17] The most likely explanation is that a carbamyl derivative is formed Stadie and O Brien[18] showed that serum proteins form carbamyl derivative although some chemists have questioned the importance of the carbamate grouping The unmistakable participation of CO in the zinc gamma globulin reaction shown by our experiments offers additional support for the existence of carbamyl groups in blood The evidence suggests also that more than one mechanism is involved in the zinc turbidity reaction The zinc carbamyl linkage does not seem to be the principal turbidity forming reaction for removal of virtually all CO still leaves a substantial zinc turbidity reading However the loss of CO_2 may cause a decrease of two

difference The prevalence of parasitic and other infections malnutrition and liver disease in the regions mentioned seemed to offer adequate explanation and it was not anticipated that Negroes in Philadelphia would share this characteristic Nevertheless we have found that the serums of healthy Negroes living in the Philadelphia area also have elevated gamma globulin concentrations That this is not a result of emigration from regions where high globulin concentrations prevail is supported by the finding that 36 per cent of those studied were native Philadelphians who had never lived elsewhere Most of the remainder had made their homes in Philadelphia many years The increase in gamma globulin was especially conspicuous when measured by the zinc sulfate turbidity method. Rawnsley *et al*[13] found that the zinc sulfate turbidity of the Negro group was 1 5 times that of the white The limits that included 95 per cent of the readings differed so widely in the Negro group that many persons would be considered abnormal if this racial difference were not taken into account It may be argued that such persons may be suffering from infections endemic among the Negro population and have high gamma globulins for this reason This seems unlikely because the contours of the Gaussian curves are similar in both groups The assumption that the entire Negro population suffers from chronic illness that stimulates gamma globulin production is implausible Involvement of only a portion of the Negro population should be reflected in distortion of the distribution similar to that pointed out in the distribution of thymol tests of blood donors There is no more evidence of this in the Negro population than in the white

Electrophoretic studies confirmed the existence of higher globulin concentrations in Philadelphia Negroes and showed that gamma globulin was responsible Concentrations of other protein components were similar in the two races with the exception of albumin which was lower in the Negroes Gamma globulin measurements by the ammonium sulfate turbidity method of de la Huerga and Popper[14] supported the electrophoretic results However the differences between the races were much less impressive when measured by these methods With the assistance of Dr William Long and Dr Raif Nassif electrophoretic studies of the protein precipitated by zinc ions were made in an attempt to learn the cause of the discrepancy The precipitates were dissolved with the aid of versene It was found that substantial amounts of beta globulin and somewhat less alpha globulin were present together with gamma globulin, in the precipitates separating from the serums of Negroes[15] Protein precipitated from serums of whites included only gamma globulin with an occasional beta globulin peak The difference in these patterns was so consistent and striking that it was possible to distinguish at a glance the racial origin of the specimen Although zinc combines with albumin beta

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or more units of turbidity which is enough to be significant for borderline values

The thymol turbidity and zinc sulfate turbidity tests have fulfilled such a distinct need in connection with the study of patients suffering from hepatitis and other diseases of the liver that the detection of aberrations in their behavior is important Both are considerably more complex than appeared likely when they were first described yet if they are to be used effectively the numerous factors that affect their behavior must be controlled rigorously to insure reliable results

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*Selection of Blood Donors: Value of Hepatic Function Tests for the Detection of Carriers of Viral Hepatitis**

ROBERT F. NORRIS, M.D., DICRAN KASSOUNY, M.D., DeWITT T. HUNTER, JR., M.D., and JOHN G. REINHOLD, Ph.D.

(Philadelphia, Pennsylvania)

The problem of detecting the asymptomatic carrier of viral hepatitis is still not solved. Since 1949 Dr. John R. Neefe and the writers have been studying the reliability of abnormal hepatic function tests as indicators of the carrier state. Although four of five proved carriers previously reported by us[1] showed persistently abnormal hepatic tests, one of our proved carriers and one of those reported by Stokes *et al.* had normal tests when first examined. It is evident that the value of hepatic function tests in detecting the carrier state of viral hepatitis will depend upon the number of those carriers having abnormal tests relative to those in whom normal results are found. Insufficient data are available to establish this ratio, since it is neither practical nor safe to employ human volunteers in a study of this magnitude.

The problem is complicated further by the higher incidence of abnormal hepatic function tests in randomly selected donors than in carefully selected healthy young adults who give no history of liver disease. In 9 per cent of donors measurements exceed the 95 per cent confidence limits in the case of one or more tests.[3] In 12 per cent measurements equal those found in many cases of established hepatic disease.[3] So-called hepatic function tests thus do not appear to be specific for liver disease and especially are not specific for the carrier state of viral hepatitis. This factor makes the rejection of all blood donors with abnormal hepatic function tests very costly and is difficult to justify unless it can be shown that in a high proportion of carriers of viral hepatitis measurements of hepatic function tests are in fact abnormal.

The purpose of this paper is to present further data on the incidence of abnormal hepatic tests in donors suspected of being carriers, to present

* This investigation was sponsored by the Medical Research Branch, Research and Development Division, Office of the Surgeon General, Department of the Army.

with a normal thymol turbidity test was selected at random for comparative measurements of the transaminase activities The serums were stored at icebox temperature Since it was reported by Karmen *et al*[8] that transaminase activity is preserved indefinitely under these conditions data in each instance are not available on the interval between time of collection of the specimen and the time of measurement In most activity was measured within three days If time of storage is an important factor in the reproducibility of results serums with normal thymol turbidity measurements serving as controls were subjected to the same environment as the serums with abnormal thymol turbidity measurements

Results

The results of hepatic function tests previously reported by Norris *et al*[4] are shown in Figure 1 Five cases which are starred were carriers proved by transmission experiments in human volunteers It is seen that in general the measurements are remarkably constant for the varying periods of observation At the time of first examination the hepatic function tests in Case 2 a proved carrier were all normal Thereafter bromsulphalein retention became progressively greater

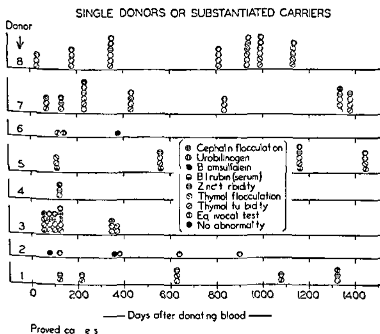


FIGURE 1

data on the persistence of abnormal hepatic function tests in donors who were subjectively healthy when first examined and to present data on the correlation of abnormal serum glutamic oxaloacetic aminopherase (transaminase) measurements with abnormal thymol turbidity tests.

Materials and Methods

Because of the high incidence of abnormal hepatic function tests in the donor population and the difficulty of follow up studies in recipients of blood transfusion, it is hard to obtain acceptable data from a study of persons who serve as multiple donors for a single recipient. The first part of the study is confined therefore to the results of hepatic function tests of donors who were proved carriers, or who were the source of a single transfusion in a recipient who suffered from an attack of viral hepatitis at a time interval following transfusion which is compatible with the incubation period of the disease. The data include 8 single donors previously reported by us[4] and 11 additional single donors whom we have examined. Also included are the single donors or substantiated carriers reported by Ginsburg *et al*[5] and Stokes *et al*. Data for a total of 4 donors are presented. Although it is not certain that each recipient acquired viral hepatitis from the blood transfusion, strong circumstantial evidence exists that each donor was a carrier of viral hepatitis at the time of blood donation.

In each instance the donor was first examined following the onset of viral hepatitis in the recipient by methods previously described.[1,4,6,7] Some donors have been examined repeatedly for several years.

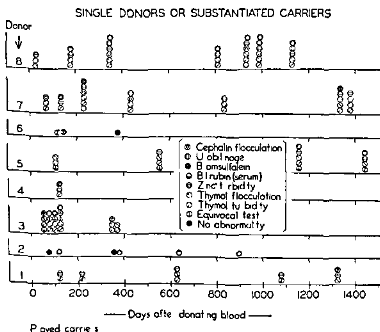
In the second part of this paper are presented follow up data for donors selected for study from a group previously reported by Fitch *et al*[3]. The donors were originally classified as "positive," "equivocal" and "negative" at the time of first examination by an arbitrary point scoring system derived from the results of hepatic function tests. The classification is also employed in the present paper. There are 9 donors originally classified as positive, [illegible] as equivocal and [illegible] as negative. Each donor was recalled at least three times for physical examination and for hepatic function tests over a period of two or more years. The results of the original examination and of the first re-examination are designated "initial test" and "initial retest" respectively. Examinations under the present program are referred to as "follow up retest."

In the third part of this study serum glutamic-oxalacetic aminopherase (transaminase) activity was measured in [illegible] persons by the method of Karmen, Wroblewski and LaDue. Of these 1 4 were selected because of a positive thymol turbidity test, [illegible] persons had an equivocal thymol turbidity test and 110 had a normal thymol turbidity test. The specimens were paired for examination. When a serum was found to show an abnormal thymol turbidity measurement a second serum of the same day

with a normal thymol turbidity test was selected at random for comparative measurements of the transaminase activities. The serums were stored at icebox temperature. Since it was reported by Karmen *et al.*[8] that transaminase activity is preserved indefinitely under these conditions, data in each instance are not available on the interval between time of collection of the specimen and the time of measurement. In most, activity was measured within three days. If time of storage is an important factor in the reproducibility of results, serums with normal thymol turbidity measurements serving as controls were subjected to the same environment as the serums with abnormal thymol turbidity measurements.

Results

The results of hepatic function tests previously reported by Norris *et al.*[4] are shown in Figure 1. Five cases, which are starred, were carriers proved by transmission experiments in human volunteers. It is seen that in general the measurements are remarkably constant for the varying periods of observation. At the time of first examination the hepatic function tests in Case 2, a proved carrier, were all normal. Thereafter bromsulphalein retention became progressively greater.



Figure

In Figure 2 and Figure 3 are shown the results of hepatic function tests in the case of 11 additional single donors whom we have examined since the previous report The results are consistent although the periods of observation are shorter The tests were normal in Cases 14 and 17 Cases 10 15 18 and 19 showed 1 abnormal test when first examined but thereafter were either normal or fluctuated between entirely normal results and one abnormal test Case 1 however was the only donor in whom 3 or more hepatic function tests were consistently abnormal Case 16 is that of a newborn child who became jaundiced 10 days after birth and remained ill with a clinical diagnosis of viral hepatitis for the following 6 weeks His mother and father had at least 1 abnormal hepatic function test whenever examined

In Figure 4 are shown the results of hepatic function tests in the case of donors reported by Ginsburg *et al*[5] and Stokes *et al* Although the methods of testing were not always identical with those employed in our laboratory *it is seen that only in Case G A a proved carrier* were all hepatic function tests normal This person also was the mother of a newborn child who suffered an attack of viral hepatitis two months after birth

If the results of hepatic function tests for each donor at the time of first

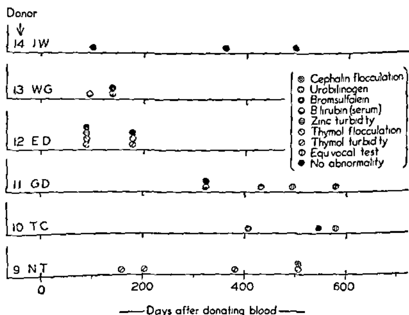


FIGURE 2

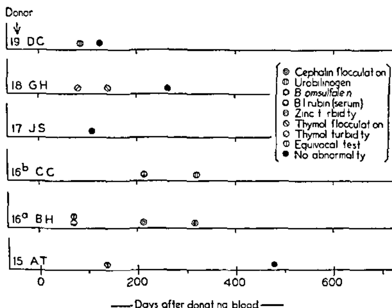


FIGURE 3

examination are combined it is seen in Table 1 that in two thirds the total scores are in the positive range When the thymol turbidity test alone is considered (Table 2) the total number of positives approximates one half By comparison as stated previously the frequency of positive and equivocal scores in the donor population as a whole is 12 2 per cent and 16 8 per cent respectively The frequency of positive and equivocal thymol turbidity tests is 6 6 per cent and 9 1 per cent respectively

Statistical analysis of the data shows that the greater incidence of positive and equivocal total scores and of positive and equivocal thymol turbidity tests among the proved and suspected carriers than among the donor population is highly significant The confidence limits for the expected incidence of positive and equivocal test among carriers are shown in Table 3 Within wide limits these figures indicate that a substantial number of carriers will have abnormal hepatic function tests but that the precise incidence cannot at present be computed from the available data

For comparison with the proved and suspected carriers of viral hepatitis

Kindly computed for us by Dr S I Askovitz

In Figure 2 and Figure 3 are shown the results of hepatic function tests in the case of 11 additional single donors whom we have examined since the previous report The results are consistent although the periods of observation are shorter The tests were normal in Cases 14 and 17 Cases 10 15 18 and 19 showed 1 abnormal test when first examined but thereafter were either normal or fluctuated between entirely normal results and one abnormal test Case 12 however was the only donor in whom 3 or more hepatic function tests were consistently abnormal Case 16 is that of a newborn child who became jaundiced 10 days after birth and remained ill with a clinical diagnosis of viral hepatitis for the following 6 weeks His mother and father had at least 1 abnormal hepatic function test whenever examined

In Figure 4 are shown the results of hepatic function tests in the case of donors reported by Ginsburg *et al*[5] and Stokes *et al* Although the methods of testing were not always identical with those employed in our laboratory it is seen that only in Case G A a proved carrier were all hepatic function tests normal This person also was the mother of a newborn child who suffered an attack of viral hepatitis two months after birth

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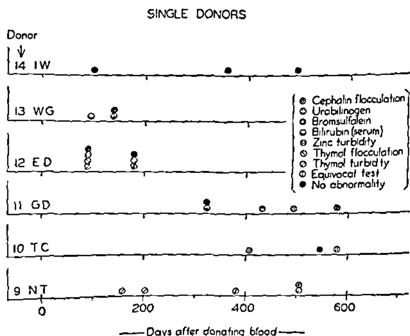


FIGURE 2

TABLE 1

TOTAL SCORES

	Negative	*Equivocal*	*Positive*
Ginsburg *et al*	—	—	2
Stokes *et al*	1	—	2
Norris *et al*	3	4	12
Totals	4	4	16

TABLE 2

THYMOL TURBIDITY ALONE

	Negative	*Equivocal*	*Positive*
Ginsburg *et al*	—	—	2
Stokes *et al*	1	—	2
Norris *et al*	9	3	7
Totals	10	3	11

TABLE 3

STATISTICAL ANALYSIS OF DATA

Observed Positive	*95 per cent Confidence Limits*	*99 per cent Confidence Limits*
Combined hepatic tests equivocal results counted as negative		
16 of 24 (66.7 per cent)	44.7 per cent to 84.4 per cent	38.6 per cent to 88.1 per cent
Combined hepatic tests equivocal results counted as positive		
20 of 24 (83.3 per cent)	62.6 per cent to 95.2 per cent	56.2 per cent to 97.0 per cent
Thymol turbidity equivocal results counted as negative		
11 of 24 (45.8 per cent)	25.6 per cent to 67.2 per cent	[illegible]0.7 per cent to 72.6 per cent
Thymol turbidity equivocal results counted as positive		
14 of 24 (58.3 per cent)	36.7 per cent to 77.9 per cent	30.9 per cent to 82.4 per cent

TABLE 4

DONOR FOLLOW-UP CLASSIFICATION

Results of Test	*Donors Originally Classified Positive (95)*			*Donors Originally Classified Equivocal (31)*			*Donors Originally Classified Negative (35)*		
Results at time of donation	P	E	N	P	E	N	P	E	N
Initial test	54	1	9	4	13	14	1	3	31
Follow-up test 1	48	18	9	9		15	1	6	8
Follow-up test 2	4	1	36	8		1	0	1	34
Follow-up test 3	33	3	3	4	10	1	0		33
Follow-up test 4	34	0	41	5	9	1	—	—	—

SINGLE DONORS OR SUBSTANTIATED CARRIERS REPORTED BY OTHERS

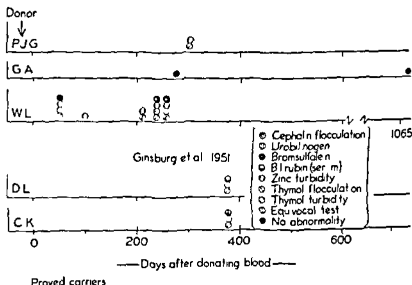


FIGURE 4

the results of repeated examinations of the groups of donors reported by Fitch *et al*[1] are shown in Table 4. Donors are grouped according to the classification by scores as positive, equivocal, and negative at the time of the initial tests. The results of scores are given for each group at the time of the retests. For the positive group, 54 of 95, or about 57 per cent, were still positive at the time of the initial retest and this number gradually fell to 34, or about 36 per cent, at the time of retest 4. Meanwhile the number of negatives increased from 9 at the time of the initial retest to 41, or nearly one half, at the time of retest 4. The number of equivocals also increased, but the trend in this subgroup was not so pronounced after the time of the initial retest.

In the case of the group originally classified as equivocal, one half or more were negative at each follow up retest. Fewer than one third were either positive or equivocal on each follow up retest.

The donors initially classified as negative showed a definite tendency to remain negative. None became consistently positive, although several were temporarily positive or equivocal.

As the study progressed, it was apparent that total scores and measure

became ill The following diagnoses were established and are charted in Table 7 according to the initial groups of the individual donors It is seen that the majority of illnesses occurred in the positive group and only a single donor in each of the equivocal and negative groups became ill with a potentially serious disease Although a number of donors are believed to be suffering from chronic hepatitis of undetermined etiology none were observed to be clinically jaundiced One donor in whom the thymol turbidity and bromsulphalein retention tests were intermittently abnormal was hospitalized elsewhere with a diagnosis of acute viral hepatitis When he was examined at this hospital before and after his illness the serum bilirubin measurements were within normal limits

The comparison of abnormal and negative thymol turbidity tests with the serum oxaloacetic aminopherase (transaminase) activities is shown in Table 8 More than one quarter of the groups having positive or equivocal thymol turbidity measurements also had excessive transaminase activities when 39 units are considered the top limit of normal but only one twelfth of the negative group had abnormal transaminase measurements Thus the frequency of abnormal transaminase activities is three times as great in the groups with abnormal thymol turbidity measurements as in the negative group If only the transaminase measurements in excess of 50 units are considered the frequency of abnormal measurements in the positive group of thymol turbidity tests is four times as great as in the negative group

In Table 9 it is seen that transaminase activities were measured in the case of 15 of the single donors presented in this paper Four or one quarter of the total exceeded 40 units Two of the 4 were in donors who also had abnormal thymol turbidity tests It should be pointed out however that transaminase activity was not measured in most of the donors until months or years after the donation of blood thought to be responsible for transmission of viral hepatitis

Statistical analysis by the Chi square method in Table 10 indicates clearly that the greater incidence of increased transaminase activities

TABLE 7

NUMBER OF CASES OF CLINICALLY ESTABLISHED DISEASES

Disease	*Group*		
	Positive	*Equivocal*	*Negative*
Diabetes mellitus	3	0	0
Peptic ulcer	4	1	0
Infectious mononucleosis	1	0	1
Disseminated lupus erythematosus	1	0	0
Rheumatoid arthritis	3	0	0
Psoriasis	2	0	0

TABLE 5

VARIATION OF DONOR SCORES

	Positive Group (95)		*Equivocal Group (31)*		*Negative Group (35)*	
	Total Scores	*Thymol Turbidity*	*Total Scores*	*Thymol Turbidity*	*Total Scores*	*Thymol Turbidity*
Consistently positive	4	12	—	—	—	—
Consistently equivocal	—	—	1	0	—	—
Consistently negative	—	—	—	—	27	31
Fluctuating	31	29	17	10	8	4
Became positive	—	—	2	2	—	—
Slow improvement	16	16	—	—	—	—
Became negative	24	38	11	19	—	—

ments of thymol turbidity fluctuated between normal and abnormal in some persons while in others the measurements remained constantly in the same range of values Thus in Table 5 in which the thymol turbidity scores are shown as well as the total scores of all hepatic tests it is seen that although 34 of 95 persons in the positive group were positive at the end of the observation period only 4 of them were consistently positive and 24 others were normal Only about one eighth of the thymol turbidity scores were consistently positive one third of them fluctuated in an erratic fashion

In the equivocal group only one score was consistently equivocal, two became positive and the majority fluctuated Fewer than one half of the thymol turbidity scores fluctuated and the majority became negative

The great majority of the total scores and the thymol turbidity scores in the negative group remained negative none became positive and only one quarter fluctuated

On physical examination the great majority of the donors showed no significant physical abnormalities The liver was palpable in one third of the cases of the positive group (Table 6) By contrast the liver was palpable in only one tenth of the negative group The incidence lay between these extremes in the case of the equivocal group

During the period of observation a number of previously healthy donors

TABLE 6

INCIDENCE OF PALPABLE LIVER

Group	*Number of Donors*
Positive	32 of 95 (34 per cent)
Equivocal	6 of 31 (20 per cent)
Negative	3 of 35 (9 per cent)

among persons having abnormal thymol turbidity measurements is highly significant This rule applies whether the thymol turbidity measurements are between the 95 per cent and 99 per cent confidence limits or whether the measurements exceed the 99 per cent confidence limits On the other hand the significance of increased transaminase activities among the single blood donors irrespective of the thymol turbidity measurements is not so well established

Discussion

The value of hepatic function tests and of the thymol turbidity test alone in detecting the carrier state of viral hepatitis in some blood donors appears to be established The results of hepatic function tests in the case of additional single donors suspected of being carriers which are presented in this paper support the concept that the number of carriers detected by these tests is significant That some carriers on the other hand have completely normal hepatic function tests is also well substantiated Even including the cases presented not enough data are currently available to define within narrow limits the ratio of one group to the other Until this ratio is clearly defined there will be reluctance on the part of many blood bank directors to reject all donors with abnormal liver function tests in view of the large number of such persons

As might be expected the results of hepatic function tests in some donors show a tendency to improve or to revert to normal In others the measurements tend to fluctuate In a large number of persons however the test results are remarkably consistent whether normal or abnormal Most of the proved and suspected carriers in the present report fall into this latter category Thus it may be assumed that some carriers having abnormal hepatic function tests will be repeatedly rejected in a screening regimen which employs hepatic function tests This situation would be found in 4 of the 5 proved carriers whom we have observed

It is not known whether those whose hepatic function tests improve over a period of time are suffering from an otherwise inapparent infection of viral hepatitis or from some other disease The cause of persistent abnormalities of hepatic function tests in many persons also is obscure More data are required to clarify these observations

The occurrence of such diseases as diabetes mellitus peptic ulcer psoriasis rheumatoid arthritis and disseminated lupus erythematosus among those donors with abnormal hepatic function tests substantiates the belief that these tests are not necessarily specific for primary disease of the liver It is apparent therefore that the clinical significance of abnormal hepatic function tests particularly in subjectively healthy persons requires further study The use of these tests for the detection of a wide variety of clinically incipient diseases is an interesting possibility

COMPARISON OF THYMOL TURBIDITY MEASUREMENTS AND TRANSAMINASE ACTIVITIES

THYMOL TURBIDITY	NUMBER TESTED	SERUM TRANSAMINASE ACTIVITY			% POSITIVE TSA		
		< 40	40 50	> 50	40 50	> 50	TOTAL > 40
POSITIVE	124	91	17	16	14	13	27
EQUIVOCAL	27	21	4	2	15	7	22
NEGATIVE	119	110	6	3	5	3	8
TOTALS	270	222	27	21	10	8	18

TABLE 8

TABLE 9

TRANSAMINASE ACTIVITIES IN SINGLE DONORS

	No	*<40*	*>40*
Positive	3	1	2
Equivocal	1	1	—
Negative	11	9	2
Total	15	11	4

TABLE 10

STATISTICAL SIGNIFICANCE OF TRANSAMINASE MEASUREMENTS

Groups Compared	*Chi Square*	*P*
Positive TT vs negative TT	9.04	<0.002
Combined positive and equivocal TT vs negative TT	7.84	<0.01
Positive TT vs equivocal TT	0.22	>0.6
Single donors vs normal controls	5.56	<0.02

Thymol turbidity

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4 Norris R F Kassouny D Reinhold J G and Neefe J R Persistence of abnormal hepatic tests in carriers of viral hepatitis *J A M A* 160 1118 1956
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The greater incidence of increased transaminase activities among those donors having abnormal thymol turbidity tests suggests that the former measurement may turn out to be a valuable addition to the list of tests for detecting the carrier state of viral hepatitis Again more data are required to ascertain whether low grade inflammatory lesions of the liver which do not cause frank clinical disease are frequently responsible for a moderate increase in the serum transaminase activity

Summary

The results of hepatic function tests in the case of 4 donors examined by us and by others are reported These donors were either substantiated carriers of viral hepatitis or were the source of a single blood transfusion for their respective recipients who subsequently suffered from an attack of viral hepatitis

Some of the donors have been examined repeatedly for several years Additionally a group of 161 donors not known to be a source of viral hepatitis in their recipients have also been examined repeatedly for two or more years The results of hepatic function tests whether normal or abnormal in most donors of both groups remained constant In some donors the results fluctuated In some with abnormal measurements there was steady improvement

When compared with the donor population as a whole the number of proved and suspected carriers having abnormal hepatic function tests and particularly abnormal thymol turbidity tests is highly significant Data are insufficient to indicate within narrow limits the proportion of carriers having abnormalities of hepatic function tests in contrast to those carriers having normal tests

The thymol turbidity test is a practical and economical method of detecting a significant number of carriers of viral hepatitis

A number of donors with abnormal hepatic function tests who felt well at the beginning of the study subsequently became ill with a variety of diseases including diabetes mellitus peptic ulcer psoriasis rheumatoid arthritis and disseminated lupus erythematosus Hepatic function tests appear to be of value in detecting incipient diseases other than primary inflammatory diseases of the liver

The incidence of increased serum glutamic oxaloacetic aminopherase (transaminase) activity is 3 to 4 times as great in subjectively healthy blood donors in whom the thymol turbidity measurements are increased as in donors in whom the thymol turbidity measurements are normal

References

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Total number of recipients studied	193
Died within first five months	12
Not available for control	20
Followed less than five months	17
Followed five months or more	144
Total number of recipients followed five months or more	144
Recipients of only one transfusion	91
Recipients of more than one transfusion	53
Total number of donors studied	600
Donors to recipients who received only one transfusion	91

FIGURE 1 Material

	D	*C*	*PDB*	*TB*	*CC*	*CR*	*TT*	*TF*
At the moment of donation	A L	1	0 11	0 41	(−)	(−)	1 3	(−)
	M G	2	0 [illegible]2	0 60	(−)	(+)	2 [illegible]	(−)
	J C	3	0 01	0 [illegible]4	(−)	(−)	3 1	(−)
At the moment of appearance of jaundice in the recipients	A L	1	0 09	0 44	(−)	(−)	2 8	(−)
	M G	2	0 12	0 36	(−)	(−)	2 8	(−)
	J C	3	0 06	0 24	(−)	(+ +)	3 9	(−)

FIGURE 2 Laboratory tests in the donors of three recipients of one transfusion who developed hepatitis with jaundice

PDB		*TB*		*CC*	
Normal	Elevated	Normal	Elevated	Negative	Positive
77	11	85	3	65	23

CR		*TT*		*TF*	
Negative	Positive	Normal	Elevated	Negative	Positive
73	15	41	47	78	10

CC CR and TF	
All negative	One or more positive
55	33

FIGURE 3 Laboratory tests in 88 donors whose recipients of a single transfusion did not develop hepatitis with jaundice

I believe has been published by others — that of putting up the ionic strength of the mixture When there is doubt about lipemia if one repeats the test with the prior addition of one drop of saturated sodium chloride that will practically abolish the reaction due to gamma globulin whereas the lipemia turbidity will be more or less unaffected I think that might perhaps be of use in the group of people with high turbidities Some of those may be lipemias and you could perhaps help them out in that way

CHARLES S DAVIDSON M D (Boston Massachusetts) I just want to comment on Dr Gyorgy s problem which is the very difficult one of how much protein a person suffering from liver disease is to be fed

DESIGNATED DISCUSSION

HECTOR DUCCI M D (Santiago Chile) Our experience is based on the study of the following material (Figure 1) in the selection of blood donors

In the group of 144 recipients satisfactorily controlled for periods from 5 to 1 months after the first or only transfusion 6 cases of hepatitis were observed (4 16 per cent) Three cases occurred among the subjects who received only one transfusion (3 3 per cent) and also three among the 53 patients who received more than one transfusion (5 66 per cent)

The results of the laboratory tests performed at the moment of donation on the donors of recipients of a single transfusion who developed hepatitis are given in Figure 2 Here are also shown the results of the tests carried out on the same donors at the time jaundice appeared in the recipients

There seems then to be no relationship between the results of the liver tests performed in the donor and the development of hepatitis with jaundice in the recipient This lack of correlation is corroborated by the results of the laboratory tests carried out at the moment of donation in the 88 donors whose recipients did not develop hepatitis with jaundice during the period of observation (Figure 3)

The analysis of the data given above reveals that three cases of hepatitis developed in 91 recipients of only one whole blood transfusion and that the three donors who induced the complication had normal serum bilirubin and negative flocculation tests Conversely in none of the recipients of blood from donors with abnormal serum bilirubin and/or positive flocculation reactions did hepatitis with jaundice occur

GENERAL DISCUSSION

N F MACLAGAN M D (London England) I was naturally most interested indeed in these two papers on the flocculation tests I admired very much Dr Reinhold s refinement of this measurement of thymol turbidity and I find that he is using it in a much more accurate way perhaps than we have ever attempted to do

I have always rather looked upon the test as a semiqualitative rather than quantitative test and there is no doubt that all the details he mentioned are of the utmost importance if importance is to be attached to very small differences in turbidity which we have not as a rule done

There is one point that I feel might possibly help and that is this question of lipemia Dr Reinhold has shown that the thymol turbidity was slightly higher after meals and occasional cases in the higher range may perhaps be due to abnormal lipemias which would not really matter from the point of view of blood donations

I wonder whether he has considered trying the method we use — which

Dr Reinhold have any information on the size of the adrenal cortex in the white race as opposed to the Negro race in Philadelphia?

John G Reinhold Ph D (Philadelphia Pennsylvania) I believe the thymol turbidity procedure is worth refining and that it is capable of giving precise results The precision that we get I would say would average about 0 2 or 0 3 unit. In terms of per cent that compares favorably with other laboratory procedures very widely used

We are of course much concerned with getting accurate results because every time we exclude a blood donor it costs money and consequently there has been that deterrent to this refinement

We have tested high ionic strength reagents such as Dr Kunkel has produced We have not tried adding just a drop of sodium chloride and that we shall attempt to do We didn t have much success with the modification that Kunkel suggested in discriminating between the two and getting results that seemed as useful

Another factor I would like to mention is that the hyperlipemia and hypobeta proteinemia that sometimes turn up in patients with liver disease — particularly in fasting patients — are measured by the thymol If one were to exclude those patients I think the test would suffer some loss of usefulness

The youngest Negroes studied by us were 18 years old These were voluntary healthy blood donors and of course there would be differences reported in African infants at birth We have no information about the adrenal cortices of our blood donors

Robert F Norris M D (Philadelphia Pennsylvania) I am glad Dr Ducci presented those negative donors of his because I do not want to give the impression Dr Maclagan that we think this has proved that we recommend in any way that anyone using these tests in a screening regimen have any feeling of compulsion I think the 24 cases we had are not enough to give precise values and I hope Dr Ducci will send me the statistics and data on those 3 cases I shall be glad to include them in any further statistical analysis we make on single donors

I don t believe there is any final answer to this at all On the one hand we have the very good and classic work of Paddock and his colleagues, suggesting that relatively large amounts of protein in alcoholics with cirrhosis are suitable Along with that goes the work in experimental animals

It has been known for many years that protein will prevent certain forms of liver disease The results from Japan of Dr Chalmers and his colleagues are equivocal I think that large amounts of protein did produce only very slight shortening of convalescence

There seems no doubt that protein is very useful and should be fed at least in adequate amounts to a person with severe liver disease unless he is suffering from signs of early hepatic coma or certainly deep coma and then I think Dr Sherlock and I would agree from our laboratories that we would omit all protein In this circumstance we are treating the brain and not the liver which I think is the most important aspect of the matter

Sheila Sherlock M D (London England) I would like to comment on Dr Gyorgy s paper

We find that in established liver disease methionine can be quite definitely toxic This toxicity can be prevented by giving wide spectrum antibiotics Presumably it is affected by changes in the bacterial flora

We have to think about more than the ordinary bacterial flora in liver disease While working last year with Dr Martini who is here we found that the bacterial content of the small intestine was considerably increased in cirrhosis one does not only have the toxic effect of methionine but also the effect of increased numbers of organisms acting on it So certainly in established liver disease nowadays I think we have no rationale for giving an amino acid that is potentially very toxic

Dr Reinhold s paper on the globulin changes in the Negro was most interesting because this year I have been at a conference in Africa where there was a great deal of talk about the increased globulins occurring in Africans It was stated that this was much more than that of an African transported from West Africa to the United States This was attributed to various things not infections particularly but malnutrition All sorts of theories were invoked much as the adrenal cortices in the Africans in Africa were different and therefore they had more response in their globulins and so on

I think it is very interesting that Dr Reinhold has shown that in presumably well nourished American Negroes the same globulin change occurs

I would like to ask him first of all how soon the globulin change occurs In the Africans it occurred within six months after birth Does

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*Storage of Blood Plasma Prevention of Virus Hepatitis by Room Temperature Storage of Pooled Plasma**

J GARRETT ALLEN MD and WYNN A SAYMAN MD
(Chicago Illinois)

In previous reports from our laboratory our principal interest in the room temperature storage of liquid pooled plasma has been to answer two questions First did any of our patients who developed homologous serum jaundice fall among those who received only plasma? Second is there any evidence that the administration of our plasma to patients also receiving our blood results in an increased attack rate of homologous serum jaundice over that encountered for the group who received our blood alone?

The answer to the first question from previous studies as well as the one reported here is No The answer to the second question has previously been No but our restudy of our plasma plus blood group along the lines of our plasma alone group as herein outlined is not yet completed and hence cannot be fully answered at this time However we have no reason to believe that our former impression for this combined group of patients is in error

We are endeavoring to establish the follow up of as many of our patients as have received plasma with or without blood In addition all blood bank records and clinical charts are being thoroughly restudied and analyzed as to the kind or kinds of transfusions each patient received This study thus far has disclosed that in our zeal to establish whether or not a transfused patient developed homologous serum jaundice too little attention was directed to the types of transfusions which the patient might have received at some time before or after his plasma transfusion during the preceding or subsequent blood bank year

To date our restudy is completed only for those patients who received only our plasma This intensive restudy of all of our records continues to disclose that none of our patients receiving only our pooled liquid

* This work is being conducted under Contract No DA 49-007-MD-93 from the Office of the Surgeon General Department of the Army

The second practice which corrupted our counts of the past was the failure to record on the patient s transfusion card all transfusions actually administered Instead all transfusion transactions were recorded in the charge book from which the patient was billed Many instances were found where the transfusion card was maintained incompletely The data in the charge book were found to be in good agreement with those recorded in the notes of the clinical chart by the nurses interns residents and attending physicians In retrospect the failure of personnel to main tain the transfusion card as efficiently as intended should have been antici pated for several reasons First during peak work loads ancillary records are likely to fail Second many of these omissions occurred after the clos-ing hours of the blood bank and on holidays when members of the house staff called for blood and/or plasma to be transfused under emergency circumstances frequently leaving only a notation in the charge book as to whom the transfusion was given

Hence during the current year literally more than a thousand man hours have been devoted toward what we now call a peeling operation paring down all the patients erroneously listed previously as having re ceived our pooled plasma only By this tedious process we have been able to reach the hard core or irreducible number for this group

The number now established for the plasma alone group cannot be further reduced However this number too will change as we continue our efforts to determine the final hard core figure for the plasma plus blood group because of (1) the incidences not yet counted for patients whose only transfusion was that of our plasma called for after blood bank hours by members of our house staff at times when the bank was unat tended and (2) the incidences for patients to whom plasma was given under emergency conditions and blood was later delivered to the bedsides but not used because the patients had already responded sufficiently well to plasma The blood was returned to the blood bank to be discarded and charges to the patient were revised but this change was not recorded on the patient s transfusion card These two reasons will necessitate the transfer of an undetermined number of patients in the other direction namely from the *plasma plus blood* group to the *plasma alone* group and possibly also some to the *blood only* group

It should be clearly stated that none of the patients transferred from the plasma only group to the plasma plus blood group were ever known to have developed homologous serum jaundice or symptoms of hepatitis Thus our basic observation that no patient who has received our pooled plasma alone is known to have developed homologous serum jaundice re mains unchanged Only the total number of patients in the plasma alone group is affected by this revision

Any transfer of patients from the blood only group or from the blood

plasma developed homologous serum jaundice However our total figures for this group of patients are in need of serious revision from those previously published The current study has disclosed many errors in our blood bank records which affect only classification as to whether a patient should be grouped in a plasma alone series or as one having received plasma with blood and/or other products

As might be expected the reclassifications have increased the plasma plus blood etc group at the expense of the plasma alone group Consequently this seriously diminishes the group whom we had previously reported as receiving only plasma This loss of course is likely to be a gain for the much larger blood plus plasma group It should be made clear that the figures to be presented here represent the absolute minimum to which the plasma alone group can be reduced and that when our final restudy is completed the numbers in this group will be larger than presented at this time Thus while these data are tentative in one way they are final in another The only subsequent revision possible for the plasma alone series when our restudy is finally completed is in the upward direction

Thus our major problem during this past year has been to establish what the hard core figure is for those patients who actually received only our pooled plasma On the surface this may seem a simple task such has not proven to be the case Indeed this one problem has been more difficult to resolve than all others including follow up

As some of the basic difficulties we have encountered may bear upon similar studies by others we believe some description as to how they came about may be desirable and perhaps useful for the problems for each patient were numerous varied and occasionally unique In principle however two practices over the years have explained most of the accounting troubles we have detected In retrospect while we have always suspected some inaccuracies from these two practices occasional spot checking failed to disclose their magnitude The first practice at fault was the annual reserialization of recipient numbers in the blood bank the first of each July from 1942 until the present beginning again each year on that date with number one This introduced the recognized problem that some patients were transfused again but during another blood bank year and were counted twice This could and did occur in the last six month period of the preceding year or first six month period of the subsequent year or both at any interval from 1 to 179 days from the July 1st date Hence double counting occurred which was a possibility and achievement that had to be eliminated In our current revision we have been able to delete all double counts save for those transfusions of plasma which were separated by periods of six months or longer on both side of the time that the plasma transfusions were administered The same review is now under way for patients who are known to have received plasma with blood

transfusion All of these have been followed or traced as indicated below

1 Personal interview and examination by physicians in the University of Chicago Clinics — 67
2 Contacted by a physician and interviewed by telephone supplemented by letter of confirmation from the patient — 64
3 Family of patient also contacted by a physician and interviewed by telephone supplemented by confirming letter — 25
4. By contact with the family physician only and supplemented by confirming letter — 25

Total 181

C *Summary of interviews* relative to symptoms of homologous serum jaundice or of hepatitis without jaundice

		Number of cases of homologous serum jaundice
1	4 patients unfollowed and untraced	?
2	104 patients followed or traced until death	0
3	181 patients followed or traced for longer than 180 days	0
Total	285 patients — no known jaundice and/or no symptoms of hepatitis	98 7 per cent
	4 patients — final outcome unknown	1 3 per cent

D *Conclusions from our current plasma only data*

1 All that may be said at this time is this We can find no evidence that recognizable signs or symptoms of jaundice developed among all of our patients who received only our plasma Ninety eight per cent of this entire group has been followed until death or for six months or longer after their plasma transfusions For six months or longer 97 8 per cent were satisfactorily followed This observation is consistent with our previous reports [1] and those of Hoxworth *et al*
2 These conclusions will be strengthened or weakened depending upon the results obtained when a similar restudy has been completed for our patients receiving our plasma plus our blood The early high death rate makes the assemblage of a large series of patients receiving plasma alone extremely difficult
3 The necessity for revision of classification is regrettable but the facts as established by the survey leave no other course The 181 patient figure for this group is now firm and represents those followed for

plus plasma series to our plasma alone group affects only the internal numerical relationships and contains none of our 88 known cases of homologous serum jaundice All cases of homologous serum jaundice have already been re examined and it is well established that they lie either in the blood only series or in the plasma plus blood group The attack rate of homologous serum jaundice for our plasma alone group still remains zero for all of our followed patients

With the above mentioned reclassifications and with the readers full realization that the final data will not be available for some months the following are the tentative figures relative to the plasma alone group of patients All donors all pools and their respective characteristics are separately tabulated on punch cards for future use

A *Donor population* Succinctly the following are the facts relative to donors comprising the plasma given these patients as nearly as we can establish at this time

1 89 patients received only plasma in which all units given were derived from a total of 68 pools of our plasma
These 68 pools were prepared from plasma derived from 76 5 donations made by 6490 donors Of the 76 5 donations 1135 represent repeat donations ranging from to times by 45 of the total donors involved in this part of our study The identity of 33 donors in the entire series is unknown
3 The mean donor exposure per patient transfused in this series is 400

B *Outcome of follow up or tracing of patients transfused*
1 Of the 89 patients receiving only our pooled plasma the final outcome of all but 4 of the 89 is now firmly established At least of the 4 are believed to be living
a 104 patients died short of six months in the University of Chicago Clinics at home or in other hospitals When death occurred elsewhere the family doctor and/or family were directly contacted
1 59 died within less than one week
2 6 died between the 7th and 31st day
3 7 died between the 30th and 61st day
4 4 died between the 60th and 91st day
5 4 died between the 90th and 1 1st day
6 3 died between the 1 0th and 151st day
7 1 died between the 150th and 181st day
Total 104

b 181 patients survived six months or longer after their last plasma

transfusion All of these have been followed or traced as indicated below

1 Personal interview and examination by physicians in the University of Chicago Clinics — 67
2 Contacted by a physician and interviewed by telephone supplemented by letter of confirmation from the patient — 64
3 Family of patient also contacted by a physician and interviewed by telephone supplemented by confirming letter — 25
4 By contact with the family physician only and supplemented by confirming letter — 25

Total 181

C *Summary of interviews* relative to symptoms of homologous serum jaundice or of hepatitis without jaundice

		Number of cases of homologous serum jaundice
1	4 patients unfollowed and untraced	?
2	104 patients followed or traced until death	0
3	181 patients followed or traced for longer than 180 days	0
Total	285 patients — no known jaundice and/or no symptoms of hepatitis	98 7 per cent
	4 patients — final outcome unknown	1 3 per cent

D *Conclusions from our current "plasma only" data*

1 All that may be said at this time is this We can find no evidence that recognizable signs or symptoms of jaundice developed among all of our patients who received only our plasma Ninety eight per cent of this entire group has been followed until death or for six months or longer after their plasma transfusions For six months or longer 97 8 per cent were satisfactorily followed This observation is consistent with our previous reports[1] and those of Hoxworth *et al*

2 These conclusions will be strengthened or weakened depending upon the results obtained when a similar restudy has been completed for our *patients receiving our plasma plus our blood* The early high death rate makes the assemblage of a large series of patients receiving plasma alone extremely difficult

3 The necessity for revision of classification is regrettable but the facts as established by this survey leave no other course The 181 patient figure for this group is now firm and represents those followed for

plus plasma series to our plasma alone group affects only the internal numerical relationships and contains none of our 88 known cases of homologous serum jaundice All cases of homologous serum jaundice have already been re examined and it is well established that they lie either in the blood only series or in the plasma plus blood group The attack rate of homologous serum jaundice for our plasma alone group still remains zero for all of our followed patients

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These 68 pools were prepared from plasma derived from 76 5 donations made by 6490 donors Of the 7625 donations 1135 represent repeat donations ranging from to 2 times by 452 of the total donors involved in this part of our study The identity of 33 donors in the entire series is unknown
3 The mean donor exposure per patient transfused in this series is 400

B *Outcome of follow up or tracing of patients transfused*
1 Of the 89 patients receiving only our pooled plasma the final outcome of all but 4 of the 289 is now firmly established At least of the 4 are believed to be living
a 104 patients died short of six months in the University of Chicago Clinics at home or in other hospitals When death occurred elsewhere the family doctor and/or family were directly contacted
1 59 died within less than one week
2 6 died between the 7th and 31st day
3 7 died between the 30th and 61st day
4 4 died between the 60th and 91st day
5 4 died between the 90th and 121st day
6 3 died between the 120th and 151st day
7 1 died between the 150th and 181st day

Total 104

b 181 patients survived six months or longer after their last plasma

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Evaluation of the Risk of Transmitting Hepatitis by the Administration of Dried Fibrinogen (Human)
(A Preliminary Report)

H D ANDERSON PH D
(Lansing Michigan)
SAM T GIBSON M D
(Washington D C.)

Following the report of Moloney Egan and Gorman[1] that afibrinogenemia may be acquired in pregnancy and the later report of Weiner Reid and Roby which described some of these conditions in detail it was clearly indicated that a demand existed for a therapeutic product rich in fibrinogen Weiner Reid and Roby[3] described a method of management which included the use of dried fibrinogen (human)

Dried fibrinogen (human) is a product prepared from human blood by a modification and elaboration of the method of Cohn *et al*[4] This preliminary report deals principally with the hepatitis risk of this product The clinical evaluation of this product for the treatment of a variety of conditions reported will be the subject of a later report Likewise the details of method of preparation will be described in another report

Through the blood program of the American National Red Cross there existed a large supply of crude Fraction I then a by product of their fractionation contracts This fraction was a rich source of human fibrinogen providing suitable methods could be employed for preparing a sterile and stable product with a low risk of transmitting the hepatitis (B) virus

Therefore it was agreed that with funds and source material provided by the American National Red Cross the Michigan Department of Health Laboratories would develop methods for reprocessing these stocks of crude Fraction I The medical staff of the American National Red Cross

This work was done at the request of the Director of the American National Red Cross Blood Program under an agreement between the American National Red Cross and the Michigan Department of Health Laboratories

six months or longer It is pointed out that the total number of patients receiving only our plasma will be increased when our entire study is completed

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1952 and to date we have distributed 45 lots representing 466 packages Each lot has varied in the content of fibrinogen per bottle the range being 0 59 Gm to 3 5 Gm The average content has been 1 60 Gm per bottle The clottable nitrogen ranged from 49 to 88 per cent with an average of 73 5 per cent

The reason that the fibrinogen content varies from lot to lot appears to be related to the freezing or freezing and drying of the crude Fraction I and the problems relating to shipping and storage before it is reprocessed Under our conditions it has been impossible to predict the degree of solubility of the crude Fraction I and consequently the amount which is removed in the clarification and filtration procedures The final product contains 6 Gm dextrose 1 4 Gm sodium citrate and 0 34 Gm sodium chloride per bottle and is isotonic when dissolved in 200 ml of distilled water Under these circumstances it is impossible to dispense a constant amount of fibrinogen without wide variation in the buffer concentration Therefore we have filled a constant volume of the buffered fibrinogen solution and recorded the fibrinogen content based on analyses for clot table nitrogen.

Results

Table 1 indicates that on the basis of 408 clinical reports received to date wherein 841 units of fibrinogen and 304 pints of blood were used this study might include ultimately 118 case reports with 466 units of fibrinogen and 8742 pints of blood Of the 408 clinical reports received 223 are complete there are 152 with six month hepatitis follow ups and 71 where the patients failed to survive through the period of hospitaliza tion None of these deaths were attributed to an unfavorable response to fibrinogen

Table 2 presents a summary of the types of cases for which fibrinogen was used It is interesting to note that 97 (73 per cent) of the cases were associated with pregnancy and that 130 (3 per cent) were diagnosed

TABLE 1

FIBRINOGEN DISTRIBUTED

Item	*Number of Cases*	*Representing Fibrinogen (Units)*	*Representing blood (Pints)*
Cases Reported	408	841	3047
Hepatitis follow ups	152	319	96
Deaths in reported cases	71	146	8 9
Follow ups due	185	3 6	1196
Cases not reported	774	1625	5700

Estimated

agreed to manage the distribution of the final products and to obtain the clinical reports and hepatitis follow ups Each bottle of fibrinogen was packaged individually together with an instruction leaflet that emphasized the potential risk of transmitting hepatitis Also included was a report form to be completed by the attending physician and forwarded to us immediately At approximately three month and six month intervals a follow up letter was sent to the physician asking that he arrange to see the patient again and report whether or not there had been any evidence of hepatitis or jaundice

Distribution depots were selected by the American National Red Cross on the basis of their geographic location as well as the willingness of the distributor to assist us in obtaining both the clinical report and the hepatitis follow up at three month and six month intervals

The Michigan Department of Health had prepared a product labeled Antihemophilic Globulin since May 1948 This was simply a sterile dry Fraction I prepared from strictly fresh plasma which had been irradiated with ultraviolet light Many reports were received wherein relatively small doses of this product appeared to correct the oozing type hemorrhage which now appears to be related to hypofibrinogenemia The reports of Tamblyn [5] and also of Coon and Hodgson [6] were the first published reports where the antihemophilic globulin was used to control this type of acquired hemorrhage The low incidence of hepatitis following the use of this product made it appear likely that larger doses of fibrinogen could be prepared by a similar method

The antihemophilic globulin had been prepared from plasma pools representing from 60 to 100 blood donors and was packaged in doses of 200 mg each The Fraction I stocks available from the American National Red Cross represented pools of not less than 2000 donors and in some cases in excess of 0 000 donors We planned to package the dried fibrinogen in doses of 2 to 3 Gm each

METHODS

Fraction I was separated by E R Squibb and Sons using the method of Cohn *et al* [4] from surplus and outdated blood arising from the blood program of the American National Red Cross The Fraction I was shipped to the Michigan Department of Health in the frozen state or as bulk dried powder This material was redissolved clarified and irradiated with ultraviolet light most lots were again reprecipitated and redissolved filtered through an antibacterial (Seitz type) filter placed in final containers frozen dried and stoppered under vacuum Tests for clottable nitrogen sterility safety and pyrogenicity were performed on final bottles selected at random

The first lot of fibrinogen was released for clinical evaluation in May

195 and to date we have distributed 45 lots representing 2466 packages Each lot has varied in the content of fibrinogen per bottle the range being 0 59 Gm to 3 5 Gm The average content has been 1 60 Gm per bottle The clottable nitrogen ranged from 49 to 88 per cent with an average of 73 5 per cent

The reason that the fibrinogen content varies from lot to lot appears to be related to the freezing or freezing and drying of the crude Fraction I and the problems relating to shipping and storage before it is reprocessed Under our conditions it has been impossible to predict the degree of solubility of the crude Fraction I and consequently the amount which is removed in the clarification and filtration procedures The final product contains 6 Gm dextrose 1 4 Gm sodium citrate and 0 34 Gm sodium chloride per bottle and is isotonic when dissolved in oo ml of distilled water Under these circumstances it is impossible to dispense a constant amount of fibrinogen without wide variation in the buffer concentration Therefore we have filled a constant volume of the buffered fibrinogen solution and recorded the fibrinogen content based on analyses for clottable nitrogen

Results

Table 1 indicates that on the basis of 408 clinical reports received to date wherein 841 units of fibrinogen and 304 pints of blood were used this study might include ultimately 118 case reports with 466 units of fibrinogen and 874 pints of blood Of the 405 clinical reports received 223 are complete there are 152 with six month hepatitis follow ups and 71 where the patients failed to survive through the period of hospitalization None of these deaths were attributed to an unfavorable response to fibrinogen

Table 2 presents a summary of the types of cases for which fibrinogen was used It is interesting to note that 297 (73 per cent) of the cases were associated with pregnancy and that 130 (32 per cent) were diagnosed

TABLE 1

FIBRINOGEN DISTRIBUTED

Item	*Number of Cases*	*Representing* *Fibrinogen (Units)*	*Blood (Pints)*
Cases Reported	409	841	3042
Hepatitis follow ups	152	319	96
Death in reported cases	71	146	8 9
Follow up due	185	3 6	1196
Cases not reported *	774	16?5	5 00

Estimated

abruptio placentae Conditions associated with long term dead fetus (missed abortion) represented 41 cases (10 per cent) Those classified under miscellaneous cases associated with pregnancy (86 cases) include threatened abortion amniotic embolism and postpartum hemorrhage of unknown etiology

Included in the group classified as postsurgical and posttraumatic were 64 cases representing 10 cases with thoracic surgery 8 cases with meta static cancer 8 cases with traumatic injury to the liver or spleen and 4 cases with esophageal varices

In the group of 39 cases which were classified as miscellaneous were Eight cases of congenital hypofibrinogenemia 8 cases associated with terminal metastatic cancer 5 cases with hepatitis or cirrhosis of the liver, 3 cases with menorrhagia and 2 with leukemia

The dosage of fibrinogen ranged from 1 to 10 Gm per case with an average of 3 2 Gm (2 1 units) Whole blood usage ranged from 0 to 104 pints per case with an average of 7 5 In 40 reports whole blood usage was not recorded and 18 reports indicated that no whole blood was used

Table 3 presents similar data for the 15 cases for which the six month hepatitis follow up has been received The general distribution of cases as well as the amount of fibrinogen and whole blood used are similar to the preceding figure Your attention is directed to the results of the hepatitis follow up Ten cases of hepatitis were reported in 152 cases which received a total of 319 units of fibrinogen and 967 pints of blood These ten cases are summarized in Table 4

The incubation period ranged between 48 and 124 days Ten lots of our fibrinogen were associated with these cases and three lots (75 77 and 86) were associated with two cases each These latter three were large lots with 79 81 and 10 bottles per lot respectively while our average lot contained 50 bottles Two of the cases also received fibrinogen pre pared by Cutter Laboratories of Berkeley California and two patients also received dried commercial plasma Each patient received whole blood the amount ranging from 1 to 3 pints Eight of the patients have recovered from hepatitis One died two days after hepatitis was diagnosed Another died following surgery for obstructive jaundice since the medi cal history did not reveal the blood and fibrinogen transfusions three months earlier

There were 18 cases reported where only fibrinogen and no blood was employed Fifteen of these cases survived and the hepatitis follow ups have been received Three of these cases received minimal exposure since the fibrinogen was used as an aid in pyelolithotomy Twelve cases in cluding 6 adults and 6 children received one or more units of fibrinogen intravenously The 6 children were diagnosed cases of congenital hypo fibrinogenemia and received 42 units of fibrinogen representing 15 to 19

TABLE 2

REPORTED CASES WHERE FIBRINOGEN WAS USED

Classification	Cases	Fibrinogen				Whole Blood		Deaths	
		Total Units	Units per Case	Total Grams	Grams per Case	Total Pints	Pints per Case	Number	Rate (Per cent)
Abruptio placentae	130	265	2.0	5.08	4.0	759	5.8	9	7.0
Premature separation	26	73	8	97.8	3.8	110	4.2	2	7.7
Dead fetus	41	85	2.1	158.7	3.9	214	5.2	4	9.8
Retained placenta	14	8	2.0	34.5	2.5	153	10.9	2	14.3
Miscellaneous with pregnancy	86	196	2.3	315.1	3.7	660	7.7	10	11.6
All cases associated with pregnancy	29	647	2.[illegible]	1126.9	3.8	1896	6.4	27	9.1
Post surgical posttraumatic	64	95	1.5	161.7	2.5	733	11.4	23	35.9
Transfusion reaction	8	22	2.8	30.3	3.8	189	23.5	2	25.0
Miscellaneous	39	77	2.0	139.4	3.6	274	5.7	19	48.9
All cases reported	409	841	2.1	1458.3	3.2	304[illegible]	7.5	71	17.4

TABLE 3

REPORTED CASES WHERE SIX-MONTH HEPATITIS FOLLOW-UPS WERE RECEIVED

Classification	Cases	Fibrinogen Total Units	Fibrinogen Units per Case	Fibrinogen Total Grams	Fibrinogen Grams per Case	Whole Blood Total Units	Whole Blood Units per Case	Hepatitis Follow-up Negative	Hepatitis Follow-up Positive
Abruptio placentae	53	104	2.0	[illegible]	3.4	405	[illegible]	52	2
Premature separation	11	30	2.7	44.5	4.0	52	4.7	11	0
Dead fetus	19	49	2.6	[illegible]	4.0	141	[illegible]	16	3
Retained placenta	5	8	1.6	11.2	2.2	34	6.8	5	0
Miscellaneous with pregnancy	30	56	1.9	93.7	3.2	[illegible]	7.0	28	2
All cases associated with pregnancy	118	247	2.1	402.2	3.5	835	7.2	112	7
Postsurgical, posttraumatic	19	76	1.4	36.9	1.9	104	5.5	16	3
Transfusion reaction	2	7	3.5	13.0	6.5	3	1.5	2	0
Miscellaneous	13	39	3.0	56.4	4.3	25	1.9	13	0
All cases reported	[illegible]	319	[illegible]	[illegible]	3.3	967	6.4	143	10

TABLE 4

SUMMARY OF HEPATITIS CASES REPORTED

Case	Incubation Period (Days)	Fibrinogen: Lot Number	Units Used	Bottles per Lot	Ppt	Reppt	Whole Blood	Plasma Units	Area
M D	112	32 35 37	3	4 79 33		✓	3	0	New York New York
B H H	61	B-51	2	36		✓	12	0	Torrington Connecticut
		Cutter s	2						
M F	—	56	1	69		✓	11	4 DC	West Reading Pennsylvania
		Cutter s	1						
H B	1 0	71	1	10?	✓		6	0	Detroit Michigan
S I	48	75	1	/9		✓	4	1 DC	Philadelphia Pennsylvania
H (Mrs)	90	75	2	9		✓	32	0	Mankato Minnesota
R M	1 4	7/	1	81	✓		1	0	Mosco Idaho
A T †	60	77	1	81	✓		8	0	London Ontario Canada
H C	85	86	3	10?		✓	3	0	Portland Oregon
R S (Mrs)	90	86 93 93	1 1 1	10? 68 68		✓ ✓ ✓	1	0	Portland Oregon

Ppt Not reprecipitated only redissolved clarified irradiated filtered filled and dried
Reppt Reprecipitated before redissolving etc
DC Dried commercial plasma
Hepatitis but no jaundice
† Epidemic of hepatitis in the community

lots The 6 adults were diagnosed as conditions associated with pregnancy and received 11 units of fibrinogen representing 6 different lots Up to this date no hepatitis has been observed in a patient who received only fibrinogen

Discussion

From the data presented it is evident that this must be considered a preliminary report These data reflect not more than 222 completed reports and represent only 465 (19 per cent) of the units of fibrinogen distributed

Under ideal conditions an evaluation of this kind should be performed in a highly selected group of teaching hospitals where careful attention to records and adequate laboratory controls are routine In this case the incidence of acute hypofibrinogenemia is relatively rare in any single hospital and it was necessary to make the product available on a nation wide basis in order to secure a reasonable experience in the four year period outlined for this study

It would also be desirable to select cases where the product under evaluation was the only possible exposure to hepatitis In the case of fibrinogen it is used under circumstances where significant quantities of whole blood are usually required Of the 408 cases which have been reported only 18 reports stated that no blood was used Forty other cases had no entry for whole blood

In spite of the intense effort to inform each physician in advance that we required an adequate clinical report and a hepatitis follow up our experience has been rather disappointing Considerable difficulty has been experienced in our efforts to obtain the clinical report forms particularly reports which contained all of the necessary information Many follow up letters and telephone calls have been necessary in order to obtain the essential information

The hepatitis follow ups have been somewhat easier to obtain once the physician and patient have been identified in our records In general the negative reports have been received slowly and have required additional reminders while the actual cases of hepatitis have been reported as soon as they were observed In spite of our efforts to make it clear that we need both the clinical report on the case and the hepatitis follow ups before releasing fibrinogen to a physician many have appeared surprised that we have asked for written reports when their patients did not contract hepatitis This prompts us to wonder if the ten cases reported to date represent the total usage of fibrinogen rather than the 152 cases where the records are complete

At the present time an exhaustive effort is being made to trace each bottle of fibrinogen distributed and to obtain complete reports for each

case treated A similar effort is being made to obtain hepatitis follow ups on each surviving case which has already been reported

Summary

A preliminary attempt has been made to evaluate the risk of trans mitting hepatitis through the use of a human blood derivative called dried fibrinogen (human) During the four and one half year period covered by this study we have prepared and distributed 45 lots repre senting 2466 bottles with an average fibrinogen content of 1 6 Gm each Only 152 complete case reports have been received which include a *six month hepatitis follow up These cases represent the total use of* 319 bottles of fibrinogen and 967 pints of blood Ten cases of hepatitis have been reported with 2 deaths Each patient who contracted hepatitis also received whole blood two also received fibrinogen prepared by *another manufacturer and 2 cases received dried commercial plasma*

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24

The Distribution of Certain Viruses in the Fractionation of Plasma

ROBERT B PENNELL PH D
(Boston Massachusetts)

Plasma consists of a mixture of numerous soluble proteins each of which presumably fulfills a specific physiological function Study of systems of separating these proteins from one another to make available the specific agent for a specific clinical requirement received great impetus in the fifth decade of this century from the needs of the armed forces of the United States At the same time and for the same reasons the use of dried whole plasma was increased tremendously In 1944 when it became evident that the possible transmission of the virus of homologous serum hepatitis by pooled human plasma was an extremely serious hazard the question as to whether this hazard could be circumvented by the use of plasma fractions rather than of whole plasma was immediately posed I propose to examine what has been learned in answer to that question

Because the method of fractionation can be all important in its influence on the presence of viruses in fractions and to permit the simplicity of using certain terms which have become standard in discussing plasma fractions a brief examination of the techniques of plasma fractionation must be made To avoid undue complexity only those systems of fractionation will be considered for which data pertinent to the subject are available

The first system of plasma fractionation to find extensive use was presented by the group at Harvard Medical School headed by the late Dr Edwin J Cohn[1] This scheme involved separation of plasma components by balancing ionic strength pH protein concentration temperature and miscible organic solvent The system underwent a number of modifications of which Method 6 became standard for separating the plasma into five major fractions A schematic representation of this method is given in Figure 1 The terms Fraction I Fraction II+III and Fraction V will be used in this discussion

For the preparation of gamma globulin from Fraction II+III Method 9 found widespread use[2] This is presented schematically in Figure 2 From

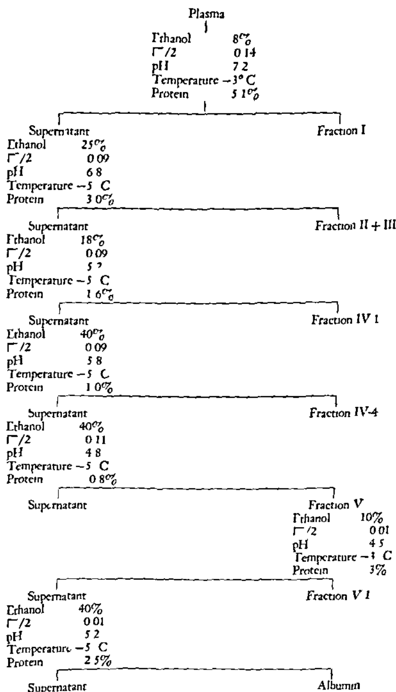
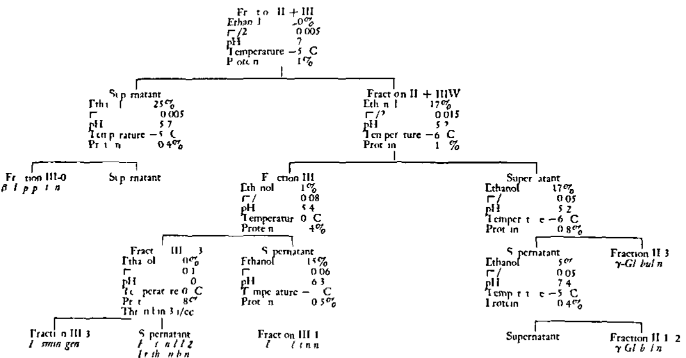


FIGURE 1 Diagrammatic representation of the preparation of normal human serum albumin — Method 6



Ficur Separ ti n of p othr mbin l lct nin plasminogen and γ gl bulins — Method 9

this method Fractions II and III will be referred to The combined procedures have come to be known as cold ethanol Methods 6 and 9

A second system emanating from the same group is dependent on the reversible stoichiometric interaction of zinc ions with plasma proteins. It has come to be known as zinc Method 1 The techniques involved require less drastic manipulation of the plasma proteins than do those of the cold ethanol systems The essentials of the zinc system are presented in Figure 3

A third system of fractionation which has been used extensively in Europe has come from the laboratory of Dr Hans Nitschmann in B rn, Switzerland (Figure 4)[3] It consists basically of cold ethanol Method 10 modified by application of the Deutsch extraction of gamma globulin[4]

Still a fourth system must be considered that of Kekwick and Mackay (Figure 5)[5] in which separation of plasma components is achieved by a system employing diethyl ether rather than ethanol

Since there has been no means of cultivating or of detecting the virus of homologous serum hepatitis in the laboratory the sole manner of accurate assessment of its distribution in plasma fractions has been the use of human volunteers which is so hazardous and costly that it has had to be severely restricted Because of these circumstances much of what is known concerning the distribution of this virus during fractionation has been gained by inference from studies with other viruses which can be more easily assessed in the laboratory

Several factors have played a part in the selection of experimental viruses for this work Ea e of study has made the bacteriophages handy tools for rapid assessment of techniques Early work[6] disclosed great variability in the stability of different viruses to chemical and physical virucidal agents Moreover the spectrum of variation might differ appreciably from agent to agent making it impossible to select a particular virus as most resistant to all techniques Thus for the evaluation by inference of the effect of any technique including fractionation on the virus of homologous serum hepatitis a variety of laboratory viruses should be studied

Certain properties of the homologous serum hepatitis virus itself have been given important consideration Immunologically there is little evidence that effective antibodies against it exist in human plasma[7 8 9] It is therefore important to choose laboratory viruses for which antibodies are unlikely to exist The persistence of serum hepatitis virus in lyophilized plasma demonstrates that one must choose a virus resistant to freezing and drying

In an early unpublished study Bird Enders and Boyd[10] added vaccinia virus tobacco mosaic virus and Theiler s mouse encephalomyelitis virus to plasma which was then fractionated by cold ethanol Method

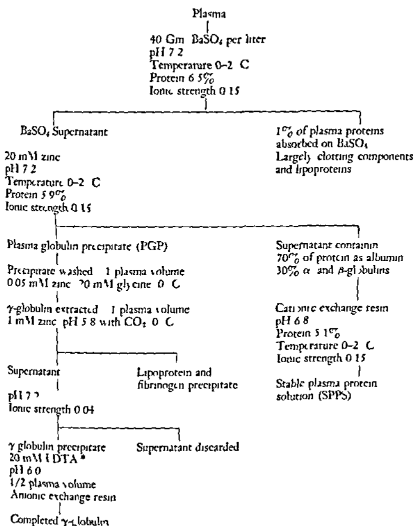


FIGURE 3 Diagrammatic representation of zinc — Method 12

* Ethylene diamine tetra acetic acid

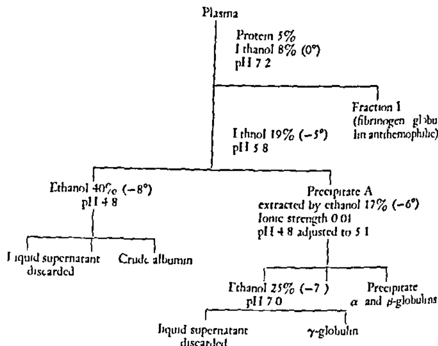


FIGURE 4 The distribution of viruses in plasma fractions

which differs from Method 9 primarily in that Fraction II is precipitated from plasma directly rather than being extracted from Fraction II and III The viruses were chosen in part because of their dissimilarity to one another No attempt was made to quantitate the data but viable virus was found in all fractions prepared namely I II III IV and V In the light of this finding an attempt was made to bring about destruction of possible virus in albumin prepared by the cold ethanol techniques Plasma known to contain the virus of homologous serum hepatitis was added to stabilized albumin and the albumin was heated for 10 hours at 60 C Gellis *et al*[11] reported that this technique destroyed the added virus as judged by infusion of the heated and of the unheated control material into human volunteers All albumin produced by cold ethanol Methods 6 and 9 subsequent to this experiment has been treated for 10 hours at 60 C prior to clinical use

Hampil Spizizen and Pennell[1] added coli bacteriophage and the virus of mouse encephalomyocarditis to plasma which was then separated into two portions one fractionated by cold ethanol Methods 6 and 9

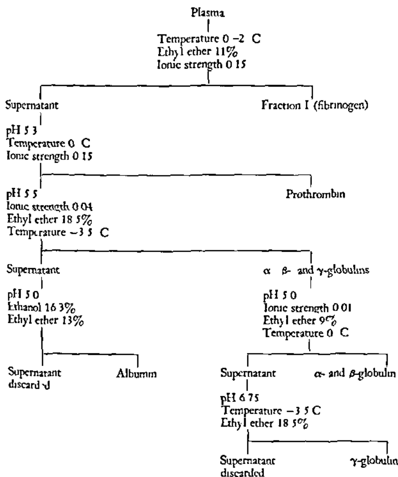


FIGURE 5 The d tribution of truse n plasma proteins

and one by zinc Method 12 Human plasma contains antibodies to neither of these viruses Both viruses were known to survive lyophilization. The data are summarized in Table 1 It will be seen that although the bulk of the two viruses was removed in the early stages of both fractionation schemes viable organisms could still be detected in the albumin produced by the cold ethanol procedure and in the stable plasma protein solution (SPPS) produced by the zinc method Moreover viable organisms were found in gamma globulin produced by both methods Early realization that serum hepatitis infection often followed the clinical use of fibrinogen (Fraction I) had led to the suggestion that, because of its presumed large size relative to the size of the plasma proteins most of the virus in plasma was removed in Fraction I These data do not bear out that thesis and would suggest that the presence of virus in Fraction I is due to the comparatively short period of exposure of the fraction to low concentrations of alcohol Chapman[13] and Smolens[14] have shown zinc Method 12 to be an excellent means of concentrating and purifying virus with essentially complete recovery The viruses which they have studied are precipitated with the plasma globulin precipitate and are not extracted from this precipitate by the washing

TABLE 1

DISTRIBUTION OF VIRUSES DURING PLASMA FRACTIONATION

	Per Cent of Original Virus Found in Fraction	
	T_2 Bacteriophage (Original Titer 2.5×10^7)	*Columbia SK Encephalomyocarditis (Original Titer 1.[illegible] $\times 10^7$)*
Zinc Fractionation — Method 12		
$BaSO_4$ Supernatant	58	28
Supernatant from 20mM zinc precipitate (SPPS *)	0.59	
SPPS after zinc removal	0.13	9
First extract of zinc precipitate (gamma globulin)	0.00009	
Gamma-globulin after zinc removal	0.000012	Trace †
Cold Ethanol Fractionation — Methods 6 and 9		
Fraction I (fibrinogen)	12	6.1
Fraction II + III	65	9[illegible]
Fraction IV-1 and IV-3, 4	[illegible]	0.8
Fraction V (albumin)	0.0000[illegible]	Trace †
Fraction II (gamma globulin)	0.00109	0.19

* Stable Plasma Protein Solution
† Too little to determine titer

procedures The present study would lend support to these findings Nevertheless if infection may be incurred from the presence of but a few viable organisms these data also suggest that no plasma fraction can be assumed to be free from a virus which may have been present in the original plasma without application of a specific sterilizing proceure

The only definitive study of the distribution of homologous serum hepatitis virus in plasma fractions has been that of Dr Roderick Murray[1] [1r] and his co workers of the Biologics Control Division of the National Institutes of Health using human volunteers In these studies a plasma pool known to contain serum hepatitis virus was fractionated both by Methods 6 and 9 in the laboratories of E R Squibb and Sons and by Method 1 in the laboratories of Sharp and Dohme Cold ethanol albumin (Table 2) given in 3 ml amounts intramuscularly produced no hepatitis but when given in full transfusion amount (100 ml containing 25 Gm) intravenously 2 of 10 volunteers were infected After being heated for 10 hours at 60 C no infection with hepatitis occurred following administration of albumin at either the 3 ml or the 100 ml level Sixteen per cent gamma globulin produced by cold ethanol Method 9 tested at 2 ml subcutaneously gave no hepatitis in 10 volunteers Gamma globulin was not tested at higher levels because it is not common practice to use it at higher levels

Albumin (SPPS) produced by zinc Method 1 (Table 3) infected 2 of 5 volunteers when tested at a level of 1 ml subcutaneously Again after having been heated for 10 hours at 60 C 1 ml subcutaneously failed to produce hepatitis in any of 10 volunteers Gamma globulin produced by Method 12 on the other hand when given to volunteers at a

TABLE 2

HUMAN VOLUNTEER STUDIES OF SERUM HEPATITIS VIRUS IN PLASMA FRACTIONS CONDUCTED BY DR JOHN OLIPHANT AND DR RODERICK MURRAY OF THE NATIONAL INSTITUTES OF HEALTH

COLD ETHANOL FRACTIONATION — METHODS 6 AND 9

Study	*Number of Volunteers*	*Size ml Route of Administration*	*Hepatitis With Jaundice*	*Hepatitis Without Jaundice*
Albumin unheated				
a small inoculum	10	3 ml I M	0	0
b large inoculum	10	100 ml I V	1	1
Albumin heated 10 hours at 60 C				
a small inoculum	10	3 ml I M	0	0
b large inoculum	10	100 ml I V	0	0
Gamma-globulin	10	2 ml 16% S C	0	0

TABLE 3

HUMAN VOLUNTEER STUDIES OF SERUM HEPATITIS VIRUS IN PLASMA FRACTIONS CONDUCTED BY DR JOHN OLIPHANT AND DR RODERICK MURRAY OF THE NATIONAL INSTITUTES OF HEALTH

ZINC FRACTIONATION — METHOD 12

Study	*Number of Volunteers*	*Size and Route of Administration*	*Hepatitis With Jaundice*	*Hepatitis Without Jaundice*
SPPS * unheated	5	1 ml S C	1	1
SPPS heated 10 hours at 60 C	10	1 ml S C	0	0
Gamma-globulin	5	4 ml 8% I V	3	2

* Stable Plasma Protein Solution

level of 4 ml of an 8 per cent solution intravenously produced hepatitis in 5 of 5 volunteers At the time of the study methods for heating gamma globulin were unknown and heated material was not studied

Another type of data may be gained by observation of recipients of plasma fractions for a period of 6 to 9 months following administration of the fraction This procedure is difficult because of the long period of observation required and because administration of any other blood product in the interim may invalidate the observation Janeway [17 18 19 20] has reported follow up studies on plasma fractions as shown in Table 4 No hepatitis infections were believed to have occurred due to 1977 injections of gamma globulin or due to 72 infusions of unheated albumin He believed one case of hepatitis in each series to be almost certainly of other origin than the fraction Fibrinogen on the other hand gave many infections There was no infection noted after administration of heated albumin

TABLE 4

FOLLOW-UP STUDIES OF PATIENTS RECEIVING PLASMA FRACTIONS PREPARED BY COLD ETHANOL METHODS 6 AND 9 (C A Janeway 1952)

Study	*Pool Size (Number of Donors)*	*Number of Injections*	*Incidence (per cent) of Hepatitis*
Fraction I (fibrinogen)	2000	47 *	17
Gamma globulin	10 000	1977	0 †
Albumin	2000		
Unheated		72 *	0 †
Heated 10 hours at 60 C		136 *	0

* Number of patients
† One case of hepatitis but evidence favor interpretation it was not due to the plasma fraction

Korns [1] has reported follow ups of 629 recipients of gamma globulin prepared by cold ethanol Methods 6 and 9 There was no incidence of hepatitis

Although used rather widely for several years thrombin (Fraction III) prepared by cold ethanol Methods 6 and 9 was not known to have given rise to hepatitis in recipients In 1953 however two production lots of this fraction were reported to have infected 15 recipients When tested critically these lots infected 10 of 15 volunteers [2, 3] It was determined that the same lot of human placental thromboplastin was u ed in the preparation of both of these thrombin lots One certainly could not exclude the placental extract as the source of the hepatitis virus particularly since this extract is used at the end of the fractionation procedure shortly before lyophilization

The final report on cold ethanol Methods 6 and 9 fractions is one of an unspecified number of injections of Fraction IV 1 resulting in four cases of hepatitis [4]

Turning to other fractionating procedures Nitschmann of the University of Bern Switzerland has prepared gamma globulin according to the method outlined earlier His colleagues reported in 1954[25] 142 injections without any known hepatitis

Fractionation of plasma by the procedure of Kekwick and Mackay has produced gamma globulin which has been reported administered to 58 recipients One of these recipients contracted hepatitis after receiving 4 ml of 16 per cent solution [26] An unspecified number of infections from thrombin prepared by this method have also been reported [5] The source of thromboplastin used in preparing this thrombin is not recorded

There are as well several reports of follow up studies on fractions to which sterilizing techniques have been applied

The fibrinogen of Janeway s tabulation had no sterilizing technique applied to it Subsequent fibrinogen prepared by the Massachusetts Biological Laboratory was treated with 2 per cent nitrogen mustard then refractionated to remove the fibrinogen from the chemical and its break down products [27] Both Janeway [14] and Diamond [5] have reported the use of this treated fibrinogen without known infection with hepatitis No precise figures have been given Dr Anderson has reported here on the use of fibrinogen which has been irradiated with ultraviolet light

In summary it is evident that the risk of hepatitis transmission is greatly minimized by the use of cold ethanol plasma fractions with the exception of Fraction I to which no sterilizing technique has been applied Nevertheless both direct and indirect evidence suggests that no fraction prepared from pooled plasma by any technique examined can be presumed to be free from the virus of homologous serum hepatitis

unless shown to be so by volunteer studies or by application of an effective sterilizing technique

Of all the fractions examined critically only Fraction II gamma globulin prepared by cold ethanol Methods 6 and 9 and probably the gamma globulin produced by Nitschmann s modification of cold ethanol Method 10 can be presumed to be free of virus contamination without specific sterilization The observation does not extend to all gamma globulin since certainly that produced by zinc Method 12 and to a much smaller extent, that produced by the Kekwick ether procedure have brought about hepatitis infection when prepared from known icterogenic plasma

Were there an explanation of this curious fact, clues of value for application to the preparation of other fractions would be provided Although the data of Hampil Spizizen and Pennell clearly show the cold ethanol procedure to destroy more of the laboratory viruses studied than does the zinc fractionating procedure contact with ethanol cannot be the entire answer Albumin produced by the cold ethanol methods is longer in contact with higher concentrations of ethanol than is the gamma globulin without destroying the last trace of hepatitis virus in Murray s study Viruses generally are less stable at low pH yet it is in albumin preparation that such low pH values are encountered rather than in gamma globulin production In the National Institutes of Health volunteer studies hepatitis infection occurred following both cold ethanol albumin and Method 12 gamma globulin injected intravenously whereas cold ethanol albumin given intramuscularly and cold ethanol gamma globulin when given subcutaneously failed to produce hepatitis It is just possible that the fact that gamma globulin is never used intravenously may play a part in its record of safety However even though the route of administration may be of some consequence when only traces of virus are present there is ample evidence that subcutaneous or intramuscular injection can produce the disease when the inoculum is ample

There thus appears to be no ready explanation of the freedom of cold ethanol gamma globulin from the virus

Plasma fractions also have definite advantage over whole plasma in the application of stabilizing and sterilizing techniques Whole plasma for example cannot be heated for 10 hours at 60 C without coagulation With the separated fraction one can select and adjust the stabilizing agent and the sterilizing technique to the fraction at hand without reference to the effect on other plasma proteins

We are making intensive studies of this nature in our own laboratory Similar work is being pursued in the laboratory of Dr L E Strong at Earlham College the laboratories of the Michigan Department of Public

Health and in the laboratories of several pharmaceutical firms In addition to albumin in its various forms beta metal binding globulin gamma globulin and plasminogen can now be heated successfully for 10 hours at 60 C. It appears likely that plasmin and ceruloplasmin can be added to this list This is the sterilizing technique most readily acceptable since it has never been shown to have failed Certainly many other techniques will reduce and probably prevent the hazard of hepatitis transmission by plasma fractions

These techniques have for the moment less certainty in that they may have been shown to fail under certain conditions or they may never have been proven by volunteer studies after application to infected plasma Nevertheless the follow up studies or studies with laboratory viruses suggest that nitrogen mustard beta propiolactone ultraviolet irradiation and cathode ray irradiation singly or in a combination may be effectively applied to the sterilization of plasma fractions

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25

The Effect of Heating for Ten Hours at 60° C on the Optical Density, Electrophoretic Distribution and Ultracentrifuge Patterns of Human Plasma Protein Solutions

DWIGHT J MULFORD PH D and
EDWARD H MEALEY
(Lawrence Kansas)

Several methods for destroying the virus of serum hepatitis have been suggested Among them are treatments using ultraviolet, high energy cathode rays heat beta propiolactone nitrogen mustard and long term storage at room temperature Information concerning the effects of these methods on plasma and plasma proteins has not been extensive If it were established that each of the methods of treatment is effective in destroying the virus then it would still be necessary to know what changes occur in the plasma especially in the plasma protein components when each method is used on plasma

We have studied the effect of 10 hours heating at 60 C on the electrophoresis and ultra centrifuge patterns and on the optical density of plasma protein solutions The present report represents such a study on solutions prepared by two different methods In both methods cold ethanol water mixtures (Cohn *et al*) were used to remove certain of the unstable plasma proteins from plasma

METHODS OF PREPARATION

Figure 1 shows the Method A procedure Normal human ACD plasma furnished by the American National Red Cross was brought to 8 per cent ethanol pH 2 ± 0 ionic strength 0 14 and temperature ± 0 5 C Fraction I contains most of the fibrinogen precipitated

The supernatant fluid following the removal of Fraction I by centrifugation was brought to 25 per cent ethanol pH 6 8 ± 0 0 ionic strength 0 12 and temperature −5 C Fraction II + III contains all the gamma globulin precipitated

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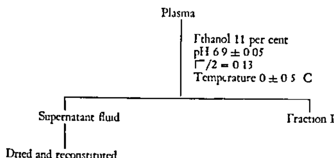


FIGURE 2 Method S

of the solutions at 550 mu as measured in the Coleman Jr Spectrophotometer Solutions prepared by Method A had an average optical density of 0 19 before heating and 0 00 after heating Solutions prepared by Method S had an average optical density of 0 365 before heating and 0 473 after heating The difference in initial optical density and the extent of the optical density change in the Method S solutions as compared to the A solutions may be partially explained by the difference in protein concentration Method A solutions were approximately 3 4 per cent in protein and Method S solutions were 4 9 per cent in protein In a separate experiment Method A solutions at 5 6 and 7 per cent protein were heated for 10 hours at 60 C Optical density changes of these solutions following the heating amounted to only 0 01 0 03 and 0 035 respectively The optical densities before heating were 0 175 0 11 and 0 245 respectively

ELECTROPHORESIS

Figure 3 shows the effect of 10 hours heating at 60 C on the electrophoretic patterns of both the Method A and Method S solutions as obtained in the Klett electrophoresis apparatus by Miss Helen Samarai of Pitman Moore Company Zionsville Indiana Method A solutions were not changed appreciably (A upper and lower pictures) Before heating they were 8 per cent albumin 13 per cent in alpha globulin and , per cent in beta globulin After heating for 10 hours at 60 C they were 83 per cent in albumin 13 per cent in alpha globulin and 4 per cent in beta globulin

The protein distribution in the Method S solutions (S upper picture) before heating was 66 per cent albumin 0 per cent alpha globulin, 4 4 per cent beta globulin and 8 4 per cent gamma globulin Some of the preparations contained a small amount of fibrinogen also Heating for 10 hours at 60 C resulted in an increase in the alpha component and a

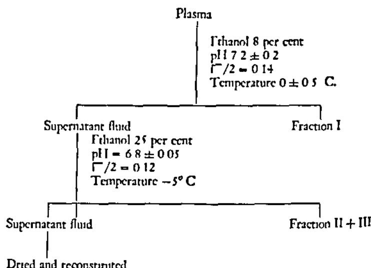


Figure 1 Method A

The supernatant fluid following centrifugation was filtered through sterilizing pads, dried from the frozen state, reconstituted to plasma volume and filtered through sterile filter pads.

Figure shows the Method S procedure. Plasma was brought to 11 per cent ethanol, pH 6.9 ± 0, ionic strength 0.13 and temperature 0 ± 0.5° C. The precipitate contained all of the fibrinogen and some of the alpha and beta globulins which normally occur in Fraction II + III.

The supernatant fluid following centrifugation was filtered through sterilizing pads, dried from the frozen state, reconstituted to plasma volume and filtered through sterile filter pads.

Optical Density

Each solution was heated in a water bath for 10 hours at 60° C. Table 1 shows the effect of heating for 10 hours at 60° C on the optical density.

Table 1

THE EFFECT OF HEATING THE PLASMA PROTEIN SOLUTIONS FOR 10 HOURS AT 60° C ON THE OPTICAL DENSITY AT 550 mμ

Method	*Protein Concentration*	*Optical Density*		*Change in Optical Density Due to Heating*
		Unheated	*Heated*	
A	3.4 per cent	0.192	0.200	0.008
S	4.9 per cent	0.365	0.473	0.108

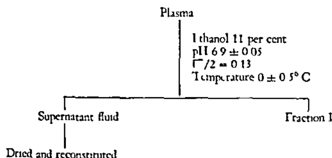


FIGURE 2 Method S

of the solutions at 550 mu as measured in the Coleman Jr Spectrophotometer Solutions prepared by Method A had an average optical density of 0 19 before heating and 0 200 after heating Solutions prepared by Method S had an average optical density of 0 365 before heating and 0.473 after heating The difference in initial optical density and the extent of the optical density change in the Method S solutions as compared to the A solutions may be partially explained by the difference in protein concentration Method A solutions were approximately 3 4 per cent in protein and Method S solutions were 4 9 per cent in protein In a separate experiment Method A solutions at 5 6 and 7 per cent protein were heated for 10 hours at 60 C Optical density changes of these solutions following the heating amounted to only 0 01 0 03 and 0 035 respectively The optical densities before heating were 0 175 0 211 and 0 245 respectively

ELECTROPHORESIS

Figure 3 shows the effect of 10 hours heating at 60 C on the electrophoretic patterns of both the Method A and Method S solutions as obtained in the Klett electrophoresis apparatus by Miss Helen Samarai of Pitman Moore Company Zionsville Indiana Method A solutions were not changed appreciably (A upper and lower pictures) Before heating they were 82 per cent albumin 13 per cent in alpha globulin and 5 per cent in beta globulin After heating for 10 hours at 60 C they were 83 per cent in albumin 13 per cent in alpha globulin and 4 per cent in beta globulin

The protein distribution in the Method S solutions (S upper picture) before heating was 66 per cent albumin 20 per cent alpha globulin 4 4 per cent beta globulin and 8 4 per cent gamma globulin Some of the preparations contained a small amount of fibrinogen also Heating for 10 hours at 60 C resulted in an increase in the alpha component and a

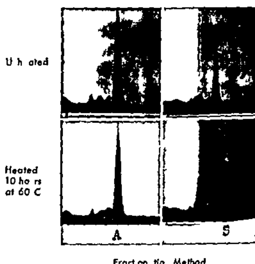


FIGURE 3 The electrophoretic diagrams of the plasma protein solutions

decrease in the albumin component (S lower picture) The beta and gamma globulin components were decreased also

ULTRACENTRIFUGE

Heated and unheated samples as prepared by Methods A and S were run at the same time in the Spinco Model E Ultracentrifuge using both cells of the rotor Each solution at 1 per cent protein was run at full speed of 59 700 rpm for 90 to 100 minutes and pictures were taken at 2 5 10 20 30 40 50 80 and 90 minutes after full speed of the rotor had been attained As the rotor approached full speed pictures were taken also at 45 000 rpm and 57 000 rpm Figure 4 shows the effect of heating the plasma protein solutions for 10 hours at 60 C on the ultracentrifuge diagrams

The pictures were taken at 0–5 0–30 and 80–90 minutes after the rotor had reached full speed The upper diagrams in each picture represent the solution heated for 10 hours at 60 C and the lower diagram the unheated control solution

A very fast moving heterogeneous component appeared in both of the solutions heated for 10 hours at 60 C as the rotor neared full speed Very little was seen in the unheated solutions (arrow 0–5 minute pictures A and S) The unheated solutions had a small amount of a fairly homogeneous material which appeared in the diagrams after the very fast moving component of the heated solutions had disappeared (arrow 20–30 minute pictures A and S)

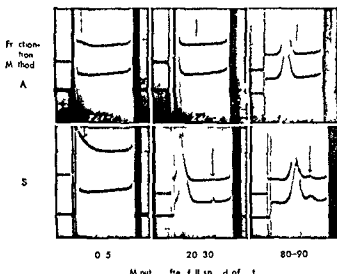


FIGURE 4 The ultracentrifugal sedimentation diagrams of the plasma protein solutions

Later in the run another component appeared in both the heated and unheated samples of the Method S solutions (arrow 80–90 minute picture S) This component was hardly discernible in the Method A solutions

The effect of 10 hours heating at 60 C on the main component of both the A and S solutions is shown in Figure 3 The greatest effect was shown by the S solutions The main component in the S solutions was markedly less than that of the unheated S solutions The main component in the heated A solutions was only slightly less than that of the unheated A solutions The area calculations of the main component of each of the heated and unheated A and S solutions show that heating the A solutions reduced the main component area by not more than 10 per cent while heating the S solutions reduced the main component area by almost 50 per cent

ANTIGENIC PROPERTIES

In some preliminary work Miss Leslie Wetterlow of the Massachusetts Department of Public Health has studied the effect of heating plasma

protein solutions at 60° C for 10 hours on their antigenic activity against horse serum hyperimmune to normal human plasma In this study the Ouchterlony double diffusion into agar technique was used Normal human plasma consistently gave eight lines Unheated plasma protein solutions as prepared by Method A gave 6 or 7 lines and heated plasma protein solutions gave only or 3 lines

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Diminishing the Risk of Hepatitis in Blood Bank Workers

SAM T GIBSON M D MADGE L CROUCH R N
and LEONARD L TAYLOR
(Washington D C)

The concept of virus hepatitis as an occupational hazard is a development of the present decade although a few references occur in the literature prior to 1950 The earliest available is that of Findlay Dunlop and Brown[1] which reports what was apparently the spontaneous infection of a laboratory worker with infective hepatitis in 1931 This worker had been handling serums from patients with infectious hepatitis Similar reports appear in the British literature in 1943 and 1944[3] In the latter year similar cases were reported by Sawyer and associates[4] in reviewing the problem of jaundice in army personnel resulting from contaminated yellow fever vaccine

The rapid growth of blood banks and the increasing amount of blood collected following World War II led to the development of blood bank work as a specialty and with this the creation of a group of people who in the course of their occupation were exposed continuously day after day to the handling of blood or plasma thus providing a far greater chance for contamination if such existed than would be encountered in the usual medical and paramedical practices In 1949 Leibowitz and others re ported a case of virus hepatitis in a blood bank worker which probably resulted from an accidental prick of her finger with needles contaminated with blood containing the hepatitis virus This was the first case that was considered an industrial hazard and was industrially compensated

In the following year Kuh and Ward[6] reviewed the literature on occupational virus hepatitis and reported 7 cases of virus hepatitis in laboratory employees who were presumably exposed to a source of in fection at work All were employed in the Cutter Laboratories and handled whole blood plasma and plasma derivatives They ranged in age from 23 to 39 and the illnesses occurred in the period December 1946 to July 1949 The case rate of hepatitis during this period was con siderably higher in the laboratory than in the surrounding community It was concluded that the handling of potentially contaminated blood or blood products was the source of infection either through cuts or

abrasions in the skin or by the oropharyngeal route To prevent a recurrence of this condition they advised against contact with blood either through the wearing of gloves or by immediate washing of hands following exposure In addition workers required by the nature of their employment to work with blood and its products have received periodic prophylactic injections of immune serum globulin since that date According to Dr Ward [7] there has not been a recurrence in the past six years

In 1951 Trumbull and Greiner [8] reported 16 cases of hepatitis in medical or paramedical personnel Only 3 however were blood bank workers

Since inauguration of the present Red Cross Blood Program in 1948 66 regional centers have been in operation at one time or another Currently there are 49 From January 1948 through June 30 1956 these 66 centers collected over 16 800 000 bottles of blood More than 7600 different employees have worked in this program during the same period If the collection testing and distribution of blood presents a hazard to the workers it would seem that cases of hepatitis would have been observed in such a large group of people during an eight year period Although this had been suspected for some time the present meeting provided the stimulus for investigating the actual number of cases

Accordingly centers now in operation were queried and the personnel records examined from all centers no longer in operation to learn the identity of employees who had had a diagnosis of hepatitis of any kind since their employment by the Red Cross Blood Program or within six months of discharge Twenty cases were reported When those persons who had had no contact with blood in any way and those who were taken ill within three weeks of employment were eliminated there remained 12 cases to be reviewed 8 nurses and 4 laboratory technicians These figures should be compared with the total number of employees in these categories nurses 3060 technicians 755 Of these cases 5 received workmen s compensation and 2 were refused The 12 cases were observed in the period February 1949 to January 1956 however 8 of them occurred in 1949–1953 2 in 1954 and only 1 each in 1955 and 1956 Are these dates significant?

Although these figures have only recently been acquired the supervisory staff of the Red Cross Blood Program has been aware of this potential problem and has continually worked to reduce the possibility of exposure to contaminated material When the program was originally set up in 1948 the blood collecting sets were prepared by personnel of the blood center They consisted of reusable needles and rubber tubing These sets were cleaned processed and sterilized by the nurses at the blood center Originally automatic finger lancets were employed to test donors hemoglobin These were later replaced by reusable needles and blades Syringes of procaine for multiple injection were employed with a different needle used for each donor

Beginning in 1950 a concerted effort was made to develop as much disposable equipment as possible particularly those items that came in contact with blood From the beginning disposable one time use bottles commercially prepared were used in all but one center With the onset of the Korean War in 1950 the reusable blood bottle was abandoned and all centers converted to vacuum disposable blood collection bottles At the same time the first plastic tubing disposable donor sets were purchased Eventually the present small lumen donor set completely disposable was developed in collaboration with the manufacturers of this equipment In the past two years we have attempted to procure a donor set with an attached disposable clamp to minimize handling of the used set by the venesectionist once the bottle of blood has been collected and the set removed from the bottle and the donor s arm With the availability of several models of donor sets equipped with completely disposable attached clamps there is little opportunity for the phlebotomist to contaminate her hands with blood from a used donor set

In 1951 when blood banks were first required to use individually sterilized hemoglobin lancets needles and syringes for procaine administration disposable substitutes were developed Triangular tipped library pins were tried first but were not found to be consistently sharp for use as lancets Taking advantage of the well established practice of taking steel pen points breaking off one nib and using the other point for a lancet the staff approached a pen manufacturer in the spring of 1951 and in collaboration with him developed a disposable hemoglobin lancet which consisted of the steel blank from which a pen point is manufactured but stamped out with only one nib and not bent to the contour of the pen staff These were prepared for the program for a year or so until disposable stainless steel finger lancets of other design were available commercially For the past two years all centers have been required to discard the finger lancet immediately upon its use to obtain blood for the hemoglobin test of a prospective donor This requirement accomplishes two things it guarantees that each donor s finger will be stuck by a finger lancet that has never been used to obtain blood before secondly it eliminates the necessity of exposing central supply room personnel to the hazard of cleaning bloody lancets The small price of the lancet makes such a practice economically feasible

Again in collaboration with manufacturers several designs of disposable procaine containers with attached needles were developed to eliminate the necessity of using individually sterilized syringes and needles Actually with the development of the disposable donor set the need for procaine has continually diminished so that in 1955–1956 less than 6 per cent of the 2 000 000 bottles of blood obtained by the program in twelve months were collected with procaine When procaine is required a small plastic tube containing 0 4 ml of procaine with an

attached needle is used and discarded Almost all items used to collect a bottle of blood are now disposable

With the exception of one case in January 1956 there have been no cases of hepatitis of any kind reported in our nursing personnel since July 195

The problem in the laboratory is another matter All specimens for identifying the blood are collected in evacuated tubes and come to the laboratory with stoppers in place It is necessary to remove these stoppers before testing the blood To date no changes in equipment design have been suggested that have made it possible to eliminate this step Strict attention to cuts abrasions and hangnails on the hands and good staff discipline are the only methods of prophylaxis at present available

In the laboratory two other duties present a potential hazard The first is cleaning glassware The employment of automatic laboratory glass washers reduces the handling of dirty equipment Twenty of the 40 centers now have this equipment installed Where washing by hand is necessary it is insisted that rubber gloves be used

The second potential source of exposure is in the disposal of red cell mass On an average — throughout the program — 1 per cent of the blood collected is either outdated or otherwise not used as whole blood This is salvaged The plasma is separated by the laboratory staff in each blood center pooled in liter bottles and shipped to commercial fractionation laboratories for the preparation of albumin gamma globulin, and fibrinogen Annually more than 00 000 bottles of blood are salvaged in this fashion It is necessary to dispose of the unusable red cell residue following the removal of plasma For public relations and sanitary reasons the bottles containing the blood cell mass cannot be placed on the city dump Most centers must remove the rubber stopper empty the cell mass rinse out the bottle and crush it or destroy the label This is a tedious and time consuming task and presents the possibility of injury by particles of broken glass and potential contamination of the hands by the blood cells

One additional step is followed in attempting to reduce the hazard to blood bank workers This step serves a double purpose in that it should also reduce the number of infected bloods unwittingly distributed for transfusion purposes For the past four years the Red Cross Blood Program has excluded as prospective donors of whole blood all persons giving a history of viral hepatitis at any time in their life or having had intimate contact with hepatitis cases in the preceding six months To obtain some idea as to the number of people eliminated by this method figures for the two week period beginning March 4 195 were procured from Red Cross blood collection and processing centers in 59 regions then in operation throughout the country[9] During that period 199 820 persons came to give donations of blood Of these 1 71 per cent

reported a past history of jaundice and 0 3 per cent an exposure to jaundice within six months the total rejection rate for these two reasons was 1 94 per cent compared with an aggregate rate of 14 6 per cent for all medical reasons in the same period

Although this elimination is in accordance with requirements of the National Institutes of Health Division of Biologics Standards some doubt exists as to how effective this method of screening by history is It does not eliminate the two groups of donors most likely to transmit hepatitis (1) carriers who according to most studies are unaware of previous clinical infection and (2) persons in the incubation phase a week or more before observing clinical symptoms of the disease and who appear to be healthy at the time of donation The exclusion of persons with an intimate exposure to hepatitis in the preceding six months rests on firmer grounds

SUMMARY

From January 1948 through June 1956 the Red Cross Blood Program procured over 16 800 000 bottles of blood collected by a total staff of approximately 7600 people 3800 of whom have been nurses and labora tory technicians During this period only 20 employees were reported to have had hepatitis In not more than 12 could it be assumed that the disease might have been related to the occupational hazard Five of these cases obtained workmen s compensation

Prophylaxis against this potential occupational hazard has consisted chiefly of (1) strict staff discipline to diminish contact with blood and ( ) the development of single use disposable blood collection equipment which is destroyed by incineration Further work is needed to improve the laboratory methods of handling specimens washing glassware and disposing of unused red blood cells

It is doubtful whether the elimination from donating whole blood of persons with a history of hepatitis is reducing the hazard of transmis sion since it fails to exclude carriers and persons in the incubation phase of the disease Exclusion for six months of persons with an intimate exposure to hepatitis is desirable The occurrence of hepatitis in employ ees of the Red Cross Blood Program has been gratifyingly low However this is no cause for relaxation of standards

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DESIGNATED DISCUSSION

JOSEPH STOKES JR MD (Philadelphia Pennsylvania) I want to mention an additional method of obtaining and preserving plasma Together with Miss Smolens Dr McGee and Dr Hunter we have been obtaining plasma by plasmapheresis in the same person biweekly over a period of one year by means of the ADL Cohn Blood Fractionator This represents an excellent method of obtaining hyperimmune plasma of serum We have done this on 23 individuals 4 of whom had been previously hyperimmunized The other 19 have been members of the Philadelphia park police — in other words they were healthy individuals

At least 3 individuals can be plasmapheresed by this method per hour The cartridges can be autoclaved while completing one plasmapheresis the second one can be started The head of the centrifuge is siliconed and all tubing is plastic tubing The blood cells are returned to the donor within approximately twenty minutes The cell cone needle is kept in the donor continuously and during the period that the red cells are being separated by the centrifuge saline is continuously infused into the donor s vessels and there is no necessity of changing the needle on this account

The advantages of course are that this system is a completely sterile system We have now performed 800 of these plasmaphereses biweekly in these 23 individuals and have had no reaction The antibodies actually have stayed up to a high level which they would not have done in the 4 hyperimmunized individuals unless their plasmapheresis had been carried out continuously according to our previous experience

So this may represent or certainly suggests that it represents a type of what we might call an immunostatic relationship in the body whereby the antibodies are kept at a high level by repeated plasmapheresis

The single plasmas that are obtained in the plastic bag can be tested easily for sterility by means of plastic tubing on one side of the bag This can be sealed off at regular intervals if one wishes by a dielectric seal and thereby the plasma can be tested for sterility or for other purposes without entering the rest of the plasma in the bag

On the other side the bag of plastic tubing extends to a needle and the plastic bag can be kept continuously at room temperature over a long period of time in the manner Dr Allen mentioned this morning It is material that has had the calcium exchanged for sodium by sulfonated resin and has no acid citrate dextrose (ACD) solution added

With the single plasmas it is as free from hepatitis virus B as the single whole blood If one wishes to type this plasma for injection into a recipient it is entirely possible to do so just as one does with whole blood

Over a period of time there has been the opportunity of testing the

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PART IV

Prevention — Chemical and Physical Agents

Moderator WILLIAM S TILLETT MD (*New York New York*)

sterility and the plasma in such a way that we can determine whether there are any alterations in the electrophoretic pattern We have not proceeded far enough to know whether this can be preserved over a long period of time but over short periods there have been no changes which are deleterious and which would prevent infusion of this into recipients

I think the other methods that of using hyperimmune plasma perhaps the exchange of infected plasma or plasma that has such materials as boric acid from poisoning or salicylate poisoning may be of more importance than the preservation of plasma itself It may be mentioned that if one calculates on a single fractionator using a fractionator about 1 hours a day over 365 days in the year 14 000 units of plasma can be obtained quite readily by means of this one machine So it does represent the possibility of getting large quantities of plasma under sterile conditions, and single plasmas probably without hepatitis virus if held at room temperature

GENERAL DISCUSSION

JAMES W MOSLEY MD (Atlanta Georgia) I would like to make one comment about the selection of blood donors

Dr Irwin A Schafer and I studied endemic hepatitis which was occurring in a penal institution in one of the southeastern states From the epidemiologic evidence it became apparent that most of the cases were probably serum hepatitis rather than infectious hepatitis and that the principal mode of transmission was illicit use of drugs by the parenteral route Poor technique in giving injections for medical and dental procedures and illicit tattooing were also factors

The institution was used by a volunteer blood agency for the collection of 60 to 00 pints of blood every six weeks We felt that in such an institution the collection of blood posed a particular danger

We would like to suggest that when a penal or similar institution is used blood donors should be very rigidly screened for the stigmata of needle use and that when hepatitis is occurring in such an institution its use as a donor source of blood should be carefully weighed against the possible risk

HANS F SMETANA MD (Delhi India) This may have nothing to do actually with this morning s discussion but the term hepatitis occurs again and again in conjunction also with hepatitis with jaundice and hepatitis without jaundice For my own information and for the information of several of the pathologists whom I know perhaps it would be well to tell how this diagnosis is made as to whether there is infectious hepatitis or just plain hepatitis or imaginary hepatitis or exactly what is meant

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Ultraviolet Radiation

JOSEPH STOKES JR MD
(Philadelphia Pennsylvania)

Because I was assigned the topic of ultraviolet radiation rather than choosing it and also in view of its introductory position on this part of the program it would seem appropriate to trace briefly the earlier history of its use in the treatment of plasma and serum for purposes of steriliza tion *and finally to state our own tentative position with respect to its* value in a combined method of treatment

It became clear to many in England and in the United States quite early in World War II that pooled plasma was responsible for the relatively high rates of serum hepatitis (hepatitis B) in the casualties from all theaters who were transfused with this pooled blood fraction Whole blood in certain theaters such as the Mediterranean when it was ob tained from men in that area apparently increased the rate of viral hepa titis considerably above that caused by pooled plasma alone This was probably because of the relatively high incidence of epidemic hepatitis and therefore blood borne virus among the men who were donating the blood during the incubation period or early phases of their disease which in many cases was inapparent or subclinical However in general the rate of hepatitis in those receiving whole blood alone has been approx imately 0 5 to 1 0 per cent in accordance with the carrier rate of the population of the United States

Our demonstration together with Dr Edwin Cohn s group in Boston that the albumin from ethanol fractionation particularly when heated to 60 C for 10 hours would not cause hepatitis (B) and its acceptance by the United States Navy permitted a chance study in which casualties in the navy and marines were receiving noninfective intravenous infusions of albumin while the casualties in the army were receiving pooled plasma Because of the size of personnel and of required supplies in the army its problems were far more complex and difficult to solve than were those of the other services The difference in rates of hepatitis among the casualties in these services who received these two blood products were particularly striking in Korea at the time of the Yalu River offensive in which the marines as well as the army participated The more recent demonstration by Murray and his associates has even more clearly shown

this field. An additional direct method of radiation developed by Sharp and Dohme uses a thin film of plasma or serum spread evenly over the outer surface of a drum which rotates into and picks up the blood fraction maintained at a constant level in a pan beneath. The ultraviolet source at a controlled distance and energy output radiates the plasma or serum from above the drum. Even were I well versed in their physical properties time would not permit a detailed discussion of the various apparatus developed for this purpose.

Oliphant and his group first used ultraviolet radiation with accurate measurement for sterilization of plasma and later were able to determine more accurately the energy applied to the plasma even though the vessel used was a 50 ml. round bottom flask of transparent fused quartz in which only about 16 ml. of serum was radiated. They used *Aerobacter aerogenes* to control the sterilizing effect of radiation. Oliphant's studies under fairly well standardized conditions and in which volunteers were used for the first time suggested that plasma and serum might be sterilized by this means.

The availability of the Habel Sockrider apparatus which permitted radiation under fairly well standardized conditions led us also to test the usefulness of such radiation in known infective serum with the injection both of control and treated batches of serum into volunteers. The 11 volunteers who received 7 ml. of irradiated serum had no evidence of hepatitis while of the 15 controls 47 per cent showed evidence of hepatitis — three with jaundice. In this study reported together with Blanchard, Hampil, Wade and Spizizen, Oliphant's cautious statements were again emphasized. We wrote: "Oliphant pointed out that the factors of titer of the icterogenic agent and the dose of serum given could possibly have an influence on the icterogenic capacity of irradiated material. Both in his experiments and in the present one there was no possibility of determining the importance of these factors." In retrospect those words of caution could well have been written in red letters. It would have been preferable to have placed this last sentence of our discussion in the summary of the paper since with so few volunteers available these and Oliphant's studies were the only ones upon which decisions concerning use of blood fractions could be made. Dr. Murray and his group later demonstrated these limiting factors of titer of hepatitis B virus and the amount of the transfusion in more extensive studies with a much larger group of volunteers on the value of ultraviolet radiation of known infective plasma. The titer of virus and the amount of the transfusion apparently had much to do with the lack of sterilization by the radiation used. In his studies with titration of the infective plasma with injection of larger amounts and with more accurate measurement of radiation sterilization was not obtained although there occurred an

that virus B is not present in the albumin obtained by the ethanol fractionation method

From the beginning of the war albumin chiefly as a 25 per cent solution was an excellent noninfective blood substitute during the relatively short period that first aid to casualties was required Whole blood could be administered a little later In these few hours the oncotic pressure is the urgent requirement rather than the other blood proteins in pooled plasma until whole blood is available The expense and relative wastefulness of producing albumin is of little significance when compared with the disability and death plus added expense of the high hepatitis rates among casualties receiving pooled plasma

The problems are presented thus in their recent historical perspective because those who were fully aware of the safety of albumin and the increasing dangers of pooled plasma were grasping at any method which might afford greater safety to the army In this complex situation of the greatest urgency the then recent demonstration of inactivation of pathogenic viruses in biological fluids by means of ultraviolet radiation appeared of far greater immediate hopefulness in prospect than now in retrospect At that time there appeared to be no other agent that afforded such simplicity of operation and such a possibility of sterilization in bulk

The two practical methods which gave greatest promise of success were those developed by Oppenheimer and Levinson as reported in 1943 and somewhat later by Habel and Sockrider The former workers used a high pressure mercury vapor compound of which a high proportion of the ultraviolet rays were of less than ooo angstrom units These rays were permitted to transverse a thin walled quartz chamber through which a thin ribbon of fluid 1 cm in width and 0 2 mm in depth passed the lamp Habel and Sockrider later demonstrated that the efficacy of the Oppenheimer Levinson method was the result of the thin nature of the film and not of the type of ultraviolet radiation used They used a different principle whereby the radiation was direct without intervening quartz A 15 watt low pressure resonance lamp was placed in the center of a revolving cylinder The cylinder was arranged at an angle of about 5 degrees from the horizontal the biological fluid to be treated was permitted to enter at one end under a semiclosed system and was rotated at about 500 to 800 rpm in a thin film on the inner surface of the cylinder while the lamp irradiated it directly as it passed to the lower end of the tube This method was later modified by these workers together with Dr Bozeman and Dr Tripp so that a tube 3 inches in diameter and 8 inches long was placed at a 15 degree angle from the horizontal and the biological material to be treated was run through at the approximate rate of 100 ml per minute The present standard Dill apparatus which was developed from this apparatus is known to all who have worked in

Table 1

EFFECT OF ULTRAVIOLET IRRADIATION BETA-PROPIOLACTONE AND THE COMBINATION OF THESE TWO AGENTS IN SERUM CONTAMINATED WITH BACTERIOPHAGE

	Original no phage particles per ml serum *	*Treatment*	*Phage counts per ml serum after treatment* 15 min	1 day	2 days	6 days
1	560×10^6	UV †	990		300	170
2	560×10^6	UV + BPL ‡		0	0	
3	494×10^6	BPL ‡	10×10^6		49×10^4	30
4	56×10^6	Control			30×10^6	9×10^6
5	100 ml of #1 after UV	BPL ‡			0	

200 ml normal human serum (Seitz filtered) per bottle

† Ultraviolet (UV) apparatus ed was standa d Dill machine Aerogenes control was complet ly k lled

‡ B-prop olactone (BPL) 3 Gm per liter In # BPL added 15 m nutes after UV

Table 2

EFFECT OF VARYING AMOUNTS OF BETA-PROPIOLACTONE FOLLOWING ULTRAVIOLET IRRADIATION IN SERUM CONTAMINATED WITH BACTERIOPHAGE

Sample	*Phage counts per ml serum* Before UV	After UV
A	$8\ 6 \times 10^6$	10/0
B	944×10^6	340

A and B divided into 4 equal portions as follows

BPL in Gm per liter		*16 hr phage counts per ml of serum*	*BPL * in Gm per liter*		*16 hr phage counts per ml of serum*
A 1	3	0	B 1	1 5	0
A 2	1 5	0	B 2	19	90
A 3	75	70	B 3	05	160
A 4	38	110	B 4	0	170

Beta p op lacto e (BPL) dded about 30 min after ultraviol t rradiation (UV)
Ae ogenes contr l 3 5 x 10 was completely k lled by UV

Results

Table 1 shows that a minute number of virus particles escaped the effects of ultraviolet irradiation or of beta propiolactone when used separately but that when the two methods were employed in sequence complete sterilization was effected

One of the major criticisms concerning the use of chemical agents for

apparent lengthening of the incubation period and reduction of the severity of the disease in the volunteers receiving irradiated plasma when compared with the controls

Once the complex machinery of production of pooled plasma in place of albumin in the great amounts necessary for the army had swung into action a crash program for sterilization by any method however inadequately tested appeared worthy of trial In such trials the increased pooling greater age of plasma increased absorption of ultraviolet energy and lack of elimination of particles all appeared to contribute to the unfavorable results reported both from the armed services and from civilian experience

This historical perspective would hardly appear worth recording here if ultraviolet light did not serve also to introduce a combined method of treatment namely the combined use of ultraviolet light and beta propiolactone which will be discussed later today but which we had a part in introducing with Dr Smolens

Orienting experiments showed that either ultraviolet treatment or beta propiolactone resulted in the death of almost all of the microorganisms The problem then would involve the inactivation of the relatively few microorganisms which survive the first type of treatment An analogy may possibly be found in the field of chemotherapy where the combination of drugs has been used to combat diseases in which minute numbers of the etiologic agent are refractory to one drug but succumb to another drug which has a mechanism of action different from the first drug It was decided therefore to utilize ultraviolet irradiation to destroy the bulk of virus particles (bacteriophage) added to serum followed by beta propiolactone in order to kill the relatively few survivors This sequence was chosen since it was not known whether ultraviolet irradiation would have any effects on the beta propiolactone although the reversal of the sequence might be perfectly satisfactory

Material and Methods

The suspending fluid used in all of the experiments was normal human serum The serums were kept frozen until the day they were to be used at which time they were thawed and filtered through a Seitz sterilizing pad and bacteriophage was added directly before treatment in order to give the concentrations shown in Tables 1 and The virus used was the T_4r coliphage furnished through the generosity of Dr S S Cohen

The ultraviolet source was the standard Dill apparatus and the method recommended by the National Institutes of Health was employed in which the rate of flow is about 250 ml per minute The beta propiolactone was supplied through the courtesy of Dr George H Mangun The treatment was carried out at room temperature

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*Inactivation of the Hepatitis Virus by High Energy Electrons**

JOHN G. TRUMP, D.Sc., and KENNETH A. WRIGHT, M.S.
(Cambridge, Massachusetts)

All forms of ionizing energy, whether ultraviolet radiation, X or gamma rays, high energy electrons, photons, alpha particles, or neutrons, are capable of modifying and, in adequate dose, destroying all forms of life.[1] In particular, ionizing energy is capable of inactivating microorganisms, including the ubiquitous though elusive hepatitis virus. The amount of ionizing energy for such inactivation depends on several factors: the nature of the energy itself, the susceptibility of the organism, its concentration in the medium, and the conditions of irradiation. Such irradiation may also exert profound effects on the normal constituents of plasma and whole blood, effects which can be minimized by proper selection of the type of ionizing energy and the conditions under which it is applied. This discussion is intended to clarify this picture and to provide a basis for the discriminating application of ionizing energy for hepatitis control.

It is possible to describe those properties of ionizing energy which best serve the purpose of inactivating the hepatitis virus and other contaminating organisms while preserving the normal constitutents of plasma and blood. Such a criterion, though elements of it may still be debated, would be helpful in evaluating the present status of plasma irradiation. The criterion should also properly include the practical and economic aspects of irradiation. On such a list of desirable or essential properties we would include the following points:

(1) The ionizing energy should be of the type which accelerates electrons within the absorber. This eliminates immediately neutrons, protons, and other nuclear particles which produce densely ionized tracks. In virus inactivation a single ionization event—or a very few—are sufficient to destroy the organism. The electron accelerating forms of ionizing energy are thus the more efficient in the inactivation of organisms and correspondingly less likely to produce adverse effects in the basic material. On this basis, the ionization from the lightly ionized tracks of

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sterilization of plasma or serum is the possibility of deleterious action on the proteins With this in mind it was thought advisable to determine the minimal concentration of beta propiolactone that would be efficacious The results presented in Table show again that small numbers of virus particles remained viable following ultraviolet irradiation but that sterilization was obtained with 1 5 Gm beta propiolactone per liter of serum when it is added after the ultraviolet treatment

We are indebted to Professor Alfred Chanutin for the electrophoretic analyses of serum before and after treatment with the combined therapy of ultraviolet irradiation and 1 5 Gm of beta propiolactone per liter The patterns were identical showing no changes in the serum proteins as determined by this technique

The results reported here based on a combined method of treatment of virus infected serum would appear to be promising and certainly worthy of further consideration particularly since the type of ultraviolet apparatus used here is already extensively in use throughout the United States and since the subsequent step of adding beta propiolactone is relatively simple

of such equipment is generally in the 50 watt to 2.5 kilowatt range. A 1 kilowatt output can deliver, assuming a 50 per cent utilization efficiency, at least 2 megarads of ionizing energy to 200 pounds of material per hour. A rad refers to an absorbed energy of 100 ergs per gram.

The penetration of such electrons depends on their energy and the relative density of the absorber, the maximum range being about 10 mm in water for each 2 million volts. Thus in a material of density δ the maximum range R in millimeters for electrons of V megavolts is $R = 5V/\delta$ (Figure 1).

The dose delivered to an absorber depends primarily on the total number of electrons per square centimeter. The effect of raising the voltage is to increase proportionately the depth to which the dose is delivered.[3] An electron current density of 4 microamperes per sq. cm. for 1 second (4 microcoulombs per sq. cm.) will deliver an average dose of 1 megarad per gram (10^8 ergs per gram) throughout a depth equal to two thirds the maximum electron range. The peak dose under these circumstances would be 1.18 megarads and the minimum dose 0.7 megarads. Since the denaturization of proteins is sensitive to the maximum dose and the persistence of organisms to the minimum radiation dose, it is desirable to minimize the spread in these values. This can be accomplished by ad-

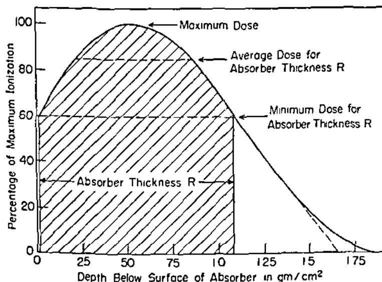


FIGURE 1 Typical distribution of [illegible] below the surface of an absorber irradiated by normally incident high energy electrons accelerated by 3.0 million volts

secondary electrons produced by X and gamma ray absorption or by high energy electrons is preferable Ultraviolet light consists of photons of low intrinsic energy the 2537 angstrom line of the mercury arc corresponds to a photon energy of about 5 electron volts and tends to be selectively absorbed It is still a low density exciter and ionizer and not excluded by this criterion

(2) The ionizing energy should be capable of penetrating to an adequate depth within the absorber so as to be independent of moderate thickness variations and inclusions it must deliver therein an approximately uniform dose Moreover it should be practical to apply the ionizing energy to the material in the liquid in the frozen and in the lyophilized state This criterion immediately rules out alpha rays and protons it comes close to ruling out ultraviolet which must be applied to films less than 100 microns thick and which has not yet been applied to irradiation of sensitive structures in the frozen state It will later be shown that irradiation in either the lyophilized or in the frozen state is virtually imperative if excessive deterioration of the blood components is to be avoided X and gamma rays and high energy electrons clearly can meet this dual criterion of irradiation in adequate depth and in any state — frozen liquid or dry

(3) The ionizing energy actually absorbed in the medium must be capable of accurate application measurement and control This is as essential in the research phase of an investigation as in the subsequent radiation processing Although all forms of ionizing energy are capable in principle of meeting this requirement there exists a wide disparity in its practical attainment The relative simplicity and accuracy of absorbed dose measurement and control for X and gamma rays and high energy electrons are attractive for processing purposes

(4) The ionizing energy must be available in adequate amount to process safely and economically the bulk quantities involved Most sources of ionizing energy appear to meet this criterion yet a wide range of processing capacity is soon apparent For example a 1000 curie cobalt 60 source of gamma rays operating continuously for over weeks would process only as much material as a 0 5 kilowatt electron accelerator could irradiate in one hour This comparison is a practical one based on present techniques of gamma ray and electron utilization Evidently in the utilization of ionizing energy it is desirable to keep in mind the processing rates and capacity required both for research and for bulk quantities

Properties of High Energy Electrons

Streams of high energy electrons from several types of machine accelerators are now available for radiation processing The output power

ceed in all directions from the source with a strong preference for the forward or continuing direction

Virus Inactivation

In an effort to determine by interpolation the electron dose required to inactivate a high concentration of the hepatitis virus 15 other viruses of various sizes and radiosensitivities were so irradiated[4] Among these were rabies polio influenza A herpes vaccinia 4 types of encephalitis mumps and Theiler s virus Irradiated at dry ice temperature 3 viruses of high titer (Lansing strain polio influenza and Jap B encephalitis) were inactivated with doses of 1 7 megarads 5 others were inactivated with doses of 3 3 megarads The remainder were given lesser doses and not completely inactivated though their reduction in titer clearly suggested that 3 3 megarads would do so Only 1 virus Theiler s in plasma at −78 C irradiated to 3 3 megarads was not completely inactivated though Theiler s in bovine albumin irradiated at − 78 C was inactivated by this dose

Further studies to improve the detail and statistical validity of this work on electron inactivation are desirable Most of the viruses were studied by Miss Julia Sullivan and Dr John Enders in Boston several by Dr William Pond and Dr Joseph Smadel at the Walter Reed Hospital in Washington D C

Viruses like bacteria and other microorganisms are believed to follow an exponential law of inactivation with the dose of ionizing energy The required inactivation dose thus depends on the initial titer the dose required to reduce the titer by the factor 2 e or 10 can be calculated from several measurements Thus the half value dose for influenza A is 120 000 rad for vaccinia is 180 000 rad

In the case of the hepatitis virus general considerations of size and radiosensitivity indicate that high doses will be required Our experimental data suggest that at least 3 megarads will be needed to inactivate the concentrations obtained from a donor at the peak stage of infectivity With the probability of lower initial titer in the donor and with the present pooling procedure a reduction in the required dose to 2 5 megarads or less may be feasible

Irradiation of Complex Materials at Low Temperature

There now exists abundant evidence that adverse side effects of irradiation on complex materials such as plasma and its several constituents can be tremendously reduced by irradiation at low temperature Thus while the irradiation of liquid prothrombin and thrombin at 0 C with a dose of 500 000 rads virtually destroys their activity irradiation at dry ice temperature produces only a 15 per cent reduction in activity at mega

justing the thickness of the absorber to a smaller fraction of the total electron range Typical distributions of dose in depth for monoenergetic electrons incident normally on an absorber are shown for 1 , 5 and 5 megavolt electrons impinging on water or unit density material (Figure 2)

The track of each high energy electron is filled with excitation and ionization events which extract energy from the primary particle and produce the chemical and biological response For high energy electrons about 8 ion pairs are found on the average per micron of path in water or similar material The ionization density increases near the end of the electron track That the ionization dose is a maximum at about one third the penetration is owing primarily to electron scattering

Although X rays are produced by the stopping of high speed electrons the efficiency of X ray production is too low to affect substantially the local electron dose distribution or to deliver a significant dose beyond the electron range With million volt electrons impinging on water less than 1 per cent of the electron energy appears as X rays These pro-

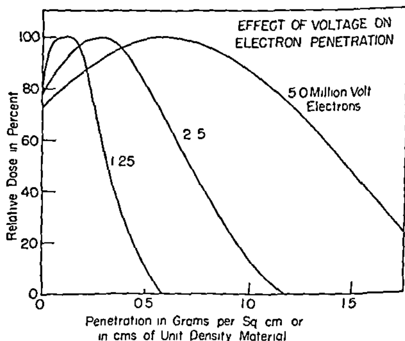


FIGURE 2 Penetration and dose distribution in water or other unit density material irradiated by 1 5 2 5 and 5 0 million volt electrons These electrons had first passed through 0 5 mm of aluminum scattering foil and 100 cm of air

room temperature There exists considerable evidence that the value of albumin as an extender is not impaired by electron doses up to 4 megarads if the irradiation is performed at reduced temperature

Adverse changes by high doses on the more complex components of plasma are to be expected and have been noted On Fraction I Dr Fred Johnson of the Cutter Laboratories has found that electron doses of 2 megarads administered at dry ice temperature produced little change in the amount of clottable protein and in its solubility with a 10 per cent reduction in clottable protein at 4 megarads However the clotting time was found to be appreciably increased and the quality of the clots was poor Similarly red blood cells glycerolized and frozen for irradiation with high energy electrons developed evidence of damage during a 5 day storage period at icebox temperature in preliminary studies by Dr Joseph P O Malley of Harvard Medical School and Dr Melvin Ketchel of the Protein Foundation It is not unlikely that further work will lead to methods of protecting against such excessive adverse effects

The combined chemical and radiation inactivation of viruses has been examined recently in co operation with Dr Joseph P O Malley at the time a medical student at Harvard in association with Dr Robert Pennell of the Protein Foundation Dr John L Oncley of Harvard Medical School and Dr John Enders of the Children s Medical Center Boston Massachusetts The combined use of material treated with beta propiolactone and irradiated with high energy electrons indicated in a preliminary way at least that these two modalities may be used in combination for inactivation of viruses each to its tolerance level for the material (Figure 4) These studies have indicated that if the virus titer is dropped by beta propiolactone alone by a factor of 10^6 and if the virus titer is dropped by electron irradiation alone by a factor of 10 then the combined use of the two would give a drop in titer of 10^1 Further studies are required to confirm this preliminary evidence

Electron Dose in Megareps	*Beta Propiolactone* Treated Samples*	*Samples Treated† only with Electrons*
0	2×10^3	$1\ 4 \times 10^3$
0	32	180
0 4	14	34
0 6	1	?
0 7	0	1

*In t l tit of T1 ph as 10 per l
†I t al tit r of T1 ph e w 1 4 × 10 per ml

(Th s tudy wa in co- p t n w th Dr J I O Mall y of th H d Med cal School)

F1 URE 4 Comb ed bet p p l t on and h h en r y lectron tr atment omp red with lectron d t o l

rads and a 30 per cent reduction at 4 megarads In Dr Wojcik's studies at the Harvard Laboratory of Physical Chemistry a dose of megarads on thrombin frozen at dry ice temperature reduced its activity by less than 30 per cent and a dose of 4 megarads diminished the activity by slightly more than 40 per cent

Dr Douglas Surgenor of that laboratory summarized the irradiation of frozen liquid stable plasma protein solution (SPPS) irradiated with electrons to a peak dose of megarads as follows Irradiation at temperatures below the freezing point of the solution has produced only barely detectable changes in the proteins of SPPS both with respect to physical characteristics and the specific protein components of the solution For example no changes were observed in the amylase choline esterase phosphatase or plasminogen levels following irradiation (See Figure 3)

With albumin studies of ultraviolet absorption spectra turbidity and viscosity were carried out at electron doses up to 5 megarads with liquid liquid with inhibitor added frozen liquid and lyophilized albumin The frozen liquid albumin gave the most favorable results since subsequent changes with incubation at 57 C for 4 hours were apparently less than with the other methods Lyophilization changed the ultraviolet absorption peak appreciably but irradiation following lyophilization did not produce further appreciable changes These studies were made with the co operation of the M I T Department of Food Technology and with Capt L R Newhouser and Dr L Rane of the Chelsea Naval Hospital

On plasma irradiated with electrons to a dose of megarads by the research staff of the Upjohn Company only minor changes in the electrophoretic pattern with the least change in the albumin peak were seen for the frozen and the lyophilized material On the other hand gross changes were obtained when the plasma was irradiated even with lesser doses at

	Electron Dose in Megareps					
	Samples Frozen			*Samples at 20° C*		
Antibodies	*2*	*4*	*6*	*2*	*4*	*6*
anti N I N5	94%	%	66%	3%	0%	0%
Coombs Sera	83	9	79	0	0	0
anti B B9033-I	100	100	100	100	64	16
anti M 7	97	91	91	3	0	0
Average of 15 Antibodies	94%	86%	87%	63%	45%	16%

(In co-operation with Dr Robert Sha Massachusetts General Hospital)

FIGURE 3 Survival of specific RBC antibodies irradiated with high energy electrons

2 megarads of electrons is 2×10^8 ergs per gram This electron dose thus delivers 20 times the energy that is now commonly used in ultraviolet radiation processing This energy ratio suggests the probably greater adequacy of electron inactivation This higher dose can be tolerated because of the ability to irradiate frozen and lyophilized material

Summary

High energy electrons are capable of inactivating viruses and destroying bacteria

Irradiation at dry ice temperature or in the lyophilized state reduces adverse effects on complex molecular structures

Four megarads may be required to inactivate the hepatitis virus from a donor in highest state of infectivity Three megarads or less may be adequate for the normal situation of infected pooled plasma

The albumin component of plasma is nearly unaffected by high electron doses delivered to frozen or lyophilized material and is probably satisfactory as an extender after such irradiation

Safety tests on irradiated plasma and tissue materials give impressive assurance against toxicity

Machine sources of high energy electrons can process the bulk quantities involved in the blood program

The comparative inadequacy of ultraviolet radiation for hepatitis control is illustrated both by its relatively low delivered energy and its inability to irradiate frozen materials on a practical basis

The desirability of further virus inactivation studies with high energy electrons for the study of protective measures for the more complex structures and for critical experiments to demonstrate electron inactivation of hepatitis infected plasma is emphasized

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Safety Tests on Irradiated Material

Substantial if not conclusive evidence has been accumulated which indicates that plasma and other tissue material may be used with safety after high doses of electron energy

In co operation with Dr Sidney Fine of the Beth Israel Hospital in Boston 2 series of tests on a total of 5 dogs were made in which dog plasma irradiated in the frozen state with 4 million rads was introduced into normal dogs on the basis of about 5 cc of irradiated plasma per kilogram No clinical response ascribable to irradiation was observed

In other dog studies normal dog blood was centrifuged and the plasma was then irradiated at room temperature by million volt electrons to a dose of megarads This was injected into nine normal dogs in the amount of 5 ml per kilogram This injection was repeated days later No side reactions were noted which distinguished the irradiated material from normal dog plasma

In several cases prothrombin irradiated to 5 megarads by electrons has been used in humans in neurosurgical procedures without noticeable adverse effects

In addition to the studies on blood derivatives and viruses work has been carried out with over 0 surgical groups on the sterilization of blood vessels and bone to be used for surgical procedures after banking for extended periods[4] These procedures involve the irradiation of frozen tissue sections to a maximum dose of megarads and hundreds of such irradiated sections have been successfully used

Energy Absorption with Ultraviolet and with Electron Irradiation

It is of interest to compare the absorbed energy delivered to plasma and other materials under the typical conditions of ultraviolet irradiation and under irradiation by some high energy ionizer such as X or gamma rays or high energy electrons

The thin film of plasma — usually 0 1 mm thick irradiated with ultraviolet radiation at room temperature — receives a dose of absorbed energy in the energy spectrum below 3000 angstroms of about 10^7 ergs per gram of material This represents that component of the delivered spectrum which contains virocidal activity with the bulk of it probably centered at the 2537 angstrom line of mercury This radiation serves some purpose in comparing the absolute absorbed energy with that delivered by a dose of 2 megarads of electron energy although it exhibits physical differences from high energy ionizers in that it is selectively absorbed by certain atomic ingredients and in that its low quantum energy (about 5 electron volts) restricts its effectiveness as an ionizer The absorbed energy with

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*Virus Inactivation with Gamma Radiation from Cobalt^{60}**

RUSSELL T JORDAN MD
(Duarte California)
LLOYD L KEMPE, PhD
(Ann Arbor Michigan)

Many attempts have been made to develop methods of sterilizing blood or its derivatives without destroying their beneficial properties Present techniques of sterilization using heat chemicals and ultraviolet light although satisfactory in some cases may result in blood or plasma which is denatured and unsafe for human use More important however plasma subjected to conditions considered sufficient for sterilization by chemicals or ultraviolet light may still contain highly resistant pathogens such as serum hepatitis (SH) virus For example Blanchard and his co workers[1] have presented evidence that ultraviolet rays may be an effective method for inactivating SH virus in plasma Although results of their studies were impressive numerous papers have appeared since reporting[2 3 4 5 6] cases of hepatitis after the use of ultraviolet irradiated plasma or serum Moreover ultraviolet irradiation does not appear to be applicable at present to whole blood which although associated with a lower incidence of hepatitis than pooled plasma appears to contribute a significant number of hepatitis virus infections[7]

Hartman and his associates[8] and Steele[9] have recommended the treatment of plasma or blood with nitrogen mustards particularly methylbis (beta chloroethyl) amine hydrochloride It is difficult to evaluate this work at present since large scale studies have not been undertaken to determine the possible toxic effects of these agents and the incidence of serum hepatitis following their use Allen and co workers[7] suggest that plasma should be stored in the liquid state without preservatives at temperatures of 80 to 96 F for 3 months or longer They consider these conditions unfavorable for the preservation of virus and maintain that serum hepatitis is rarely encountered after storage in this manner Others[10]

* Supported by Grant No C- 641 United States Public Health Service

preparations were exposed to cobalt⁶⁰ radiations from 1 to 4 5 million rep Frozen crude and partially purified virus suspensions diluted in 0 0₁ Molar phosphate buffer to contain the same number of LD ₀ and whole brain preparations were irradiated as described Samples of virus were removed after periods of irradiation and virus titer was determined by intracerebral injection in mice

Results of a typical experiment are seen in Table 1 Crude suspensions of SLE virus were more resistant to gamma ray inactivation than were partially purified suspensions By way of comparison crude virus was reduced one hundredfold after exposure to 1 0 million rep while partially purified samples receiving the same dosage were inactivated approximately ten times faster Partially purified SLE virus was no longer infectious after 3 0 million rep while crude virus required an additional 1 0 million rep before suspensions lost their pathogenicity for mice

Results of Table 1 are presented graphically in Figure 1 Points on these curves are averages of virus dilution expressed as logarithms of LD ₀ It may be seen from this figure that the slopes of the curves for whole brain and crude virus appear to be parallel and from this it may be inferred that the inactivation rates for SLE virus in crude suspension and whole brain are essentially the same

Purified virus on the other hand was more susceptible to the lethal effects of gamma radiation and required a considerably smaller dose for complete inactivation These data suggest that purification of virus removed some substance which protected the virus particle from the lethal effects of gamma rays

The rates of inactivation for crude and partially purified virus suspensions for all viruses studied are seen in Figure When the surviving virus fractions or LD ₀ are plotted on a logarithmic scale the points are found to lie approximately on a straight line showing that within error of the experiment the surviving fraction is an exponential function of the dose It should be pointed out that although error of assessment of virus

INACTIVATION OF ST LOUIS ENCEPHALITIS VIRUS
USING GAMMA RADIATION

VIRUS PREPARATION	UNIRRADIATED CONTROLS	rep* RADIATION LEVELS IN MILLIONS							
		10	15	20	25	30	35	40	45
WHOLE BRAIN	7 7	5 2	3 7	3.0	2.6	2.1	1.5	1 2	0
CRUDE VIRUS	6 0	40	30	24	20	15	12	0	0
PURIFIED VIRUS	6 0	3.2	24	17	12	0	0	0	0

ROENTGEN EQUIVALENT PHYSICALS one rep represents an energy absorption dose of 93 ergs/gram

**ALL FIGURES represent logarithm of LD_{50}

TABLE

have indicated that the SH virus can be inactivated in human albumin by heating at 60 C for 10 hours

A completely satisfactory procedure for the inactivation of the etiologic agent of serum hepatitis has not yet been found The need therefore for obtaining better methods of inactivating viruses in blood or its derivatives indicates the desirability of exploring more efficient means of virus inactivation

Since a suitable laboratory animal for SH virus is unavailable these studies deal with the effect of gamma radiation on 6 other animal viruses in vitro

Materials and Methods

Experiments were designed to determine the dosage necessary to inactivate viruses tested when irradiated in whole brain or in suspension Since preliminary studies suggested a relationship between size of viruses and their resistance to inactivation by ionizing radiations viruses selected covered a range of particle size

Viruses Viruses used were the Lansing strain of poliomyelitis 22–7 mμ [11] Hubbard strain of St Louis encephalitis (SLE) 0–30 mμ [1] California strain of western equine encephalomyelitis (WEE) 53 mμ [13] and the Armstrong 1166 neurotropic strain of vaccinia virus 5 mμ [14]

Virus suspensions were prepared from infected brains of 3 to 4 week old Swiss mice Suspensions were then centrifuged at low speed to obtain crude virus (CV) with subsequent high speed centrifugation and Seitz filtration to obtain partially purified virus (PPV) [1] Samples were irradiated from 5 to 4 hours during which time they were kept frozen with dry ice at approximately − 7 °C

Method of Irradiation All samples for a given experiment were exposed to gamma radiation from a cobalt^{60} source until the required dosage in roentgen equivalent physicals (rep) was attained One rep is defined for tissue in air represents an energy absorption dose of 93 ergs per gram Ferrous ferric dosimetry was used for calibration The calibration solution was placed in vials similar to those used for virus preparations and readings were based on oxidation of 15 4 micromoles of ferrous ions per liter per 1000 rep After the required exposure samples were removed and immediately tested for presence of virus by intracerebral titration in mice All LD_{50} titers were determined by the method of Reed and Muench [16]

Results

Inactivation of Viruses by Gamma Radiation As a first step toward studying the effect of gamma rays on animal viruses samples of virus

preparations were exposed to cobalt[60] radiations from 1 to 4 5 million rep Frozen crude and partially purified virus suspensions diluted in 0 05 Molar phosphate buffer to contain the same number of LD 50 and whole brain preparations were irradiated as described Samples of virus were removed after periods of irradiation and virus titer was determined by intracerebral injection in mice

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CRUDE VIRUS	6 0	40	30	24	20	15	12	0	0
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TABLE

INACTIVATION OF
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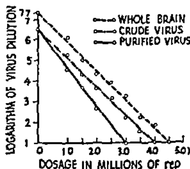


FIGURE 1

activity may sometimes be rather large there appear to be no constant deviations from exponential survival as distinct from random variations

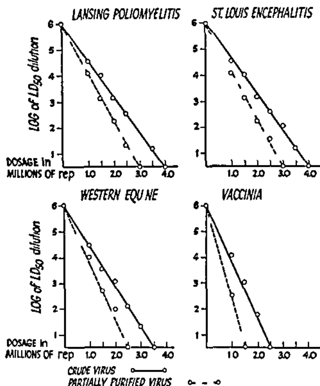
The relationship between virus size and dose of gamma radiation required for inactivation is seen in Table The data show that crude virus suspensions of SLE and polio virus required the same dosage of radiation before they were inactivated while vaccinia virus was rendered noninfectious by a significantly smaller dose Data obtained with partially purified suspensions suggest that smaller virus particles require greater doses of radiation for inactivation than do larger virus particles It appears that the differential in size between SLE and polio virus is not sufficient to make a difference in the dose of cobalt radiation needed for inactivation

Of the partially purified virus suspensions tested SLE and polio virus with diameters of 0–30 mμ required 3 0 million rep for complete inactivation while the intermediate WEE virus required a slightly smaller dose Vaccinia the largest virus used was inactivated with half the radiation required for the smallest viruses

Effect of Gamma Radiation on Human Plasma

With the demonstration that gamma radiation is an effective method of inactivating certain animal viruses experiments were designed to determine the effect of ionizing radiation on human plasma Sixteen units each containing 500 ml of pooled plasma were exposed to various levels of radiation Samples were irradiated at room temperature and tested for toxicity by intracerebral and intraperitoneal injection in mice and rabbits

To determine the radiation dose necessary to inactivate SLE virus in plasma o per cent suspensions of virus infected mice brains were pre



FIGURE

pared using plasma as a diluent St Louis encephalitis virus was used as the test organism since it approximates the size of SH virus.[13] Tubes containing virus seeded plasma were attached to their corresponding units and irradiated at the same time The data in Table 3 indicate that levels of radiation required to inactivate SLE virus do not render the plasma toxic for mice or rabbits Little or no change occurred in pH following irradiation and no sediment or apparent physical change occurred in any of the units tested The intravenous administration of gamma irradiated plasma into 16 patients ranging from to 60 years of age produced no untoward reactions There was no elevation in temperature during or after

Appreciation expressed to Dr A Knudson Jr and Dr S Rappaport Department of Pediatrics, and Dr H E Rattunde Department of Medicine City of Hope Medical Center for their assistance in the clinical aspects of this portion of the study

RELATION BETWEEN VIRUS SIZE AND INACTIVATION DOSE OF GAMMA RADIATION

VIRUS	DIAMETER IN mμ	C.V	PPV
POLIOMYELITIS	22 27	40*	30
ST LOUIS	20 30	40	30
WESTERN EQUINE	53	35	25
VACCINIA	225	25	15

FIGURES REPRESENT rep IN MILLIONS
C.V CRUDE VIRUS
PPV PARTIALLY PURIFIED VIRUS

TABLE 2

the administration of plasma in any of the species tested It is interesting to note that even though plasma received approximately twice the amount of radiation required for inactivation of St L virus it nevertheless remained nontoxic for patients and laboratory animals Studies on irradiated plasma subjected to paper electrophoresis and analyzed in the Spinco Analytrol indicate that the per cent of albumin decreases as irradiation increases

EFFECT OF GAMMA RADIATION ON POLIOMYELITIS IMMUNE GLOBULIN

Studies by Janeway [17] suggest that antihemophilic fractions of human plasma may sometimes transmit viral hepatitis This has raised the question as to whether other products of human plasma fractionation now being used in the treatment of certain diseases may also contain SH virus Hepatitis has followed the administration of Cohn s Fraction I [18] and the use of human thrombin derived from pooled plasma prepared from Cohn s Fraction III [1] Serum hepatitis has also been reported in children who received Fraction IV prepared from pooled postpartum plasma used in the treatment of rheumatoid arthritis Although recent studies [1] have confirmed earlier impressions [1] that albumin and gamma globulin fractions are unlikely to play a role in the transmission of homologous serum hepatitis others [3] conclude that gamma globulin is probably not entirely free of icterogenic virus Aside from the possibility that gamma globulin may carry SH virus it was considered of interest to determine the effect of radiation on antibodies found in poliomyelitis immune globulin Concentrated poliomyelitis immune globulin (Sharp and Dohme) containing approximately 165 mg of globulin per ml was titrated in HeLa cell cultures against Type I (Mahoney) and Type II (MEFI) strains of poliomyelitis viruses Tenfold serial dilutions of immune globulin were made

The authors wish to express their appreciation to Dr Pamela H Byatt D part

EFFECT OF COBALT[60] RADIATION ON HUMAN PLASMA

PROPERTIES STUDIED		NON IRRADIATED CONTROLS	MEGA rep GAMMA RADIATION			
			1 0	2 0	3 0	4 0
TOXICITY	MICE	0/6*	0/6	0/6	0/6	0/6
	RABBITS	NR	NR	NR	NR	NR
	MAN	NR	NR	NR	NR	NR
	pH	7 83	7 82	7 80	7 82	7 87
CHANGE IN % ALBUMIN ELECTROPHORESIS		40	36	31	26	20
SLE SEEDED PLASMA IC IN MICE LD_{50} 10 7 1		6/6	4/6	2/6	0/6	0/6

ROENTGEN equivalent physicals in millions
PLASMA given IC and IP to laboratory anim ls
SLE ST Louis Encephalitis virus

NUMERATORS number of deaths
DENOMINATOR no. of mice used
**AVERAGE of pool

TABLE 3

with balanced salt solution and seeded either with 100 $TCID_{50}$ of Type I or 150 $TCID_{50}$ Type II polio virus Eight tubes were used for each dilution and titers were recorded as the highest dilution of globulin which neutralized the calculated dose of virus Results with preirradiated and postirradiated samples are seen in Table 4 It is apparent that reasonably high doses of radiation had no detectable effect on the neutralizing capacity of globulin against the viruses tested Paper electrophoresis analysis of irradiated samples indicated a decrease in globulin concentration with an increase in radiation It appears that either the reduction in globulin is not sufficient to be detected by virus neutralization in tissue culture or that the fraction of globulin reduced does not contain significant amounts of specific neutralizing antibodies for the viruses tested

UTILIZATION OF GAMMA RADIATION FOR THE STERILIZATION OF BONE HOMOGRAFTS

With the advent of improved surgical techniques in the expanding field of orthopedic surgery the use of bone homografts is receiving increasing clinical application Although the incidence of virus hepatitis following transplantation of tissue homografts is not known serum hepatitis has been reported following the use of refrigerated bone [4] The supply of homografts obtained usually from necropsy may be contaminated with bacteria or latent viruses Moreover the availability of tissues to be used for surgical transplant is limited and the supply of bone segments must be preserved for eventual use The widespread use of freeze drying for the

ment of Infectious Diseases University of California Medical School at Los Angeles for titration of the poliomyelitis immune globulin and to Dr A F Rasmussen for his helpful suggestions during the course of the problem

EFFECT OF COBALT 60 RADIATION ON NEUTRALIZING ANTIBODIES AGAINST POLIOMYELITIS VIRUS IN IMMUNE GLOBULIN

VIRUS	NEUTRALIZING TITER BEFORE IRRADIATION	MEGA rep* GAMMA IRRADIATION			
		1 0	2.0	3 0	4 0
*MAHONEY TYPE I	1 100	1 100	1 100	1 100	1 100
**M.E.F I TYPE II	1 500	1 500	1 500	1 500	1 500

ROENTGEN equivalent physicals in millions
**DILUTION endpoint in HeLa cells
100 $TCID_{50}$ } VIRUS INOCULUM
**158 $TCID_{50}$

TABLE 4

preservation of blood derivatives is now being extended to the preservation of skin bone and blood vessels [3] Unfortunately methods which preserve tissues to be used for homografts may also preserve viruses The unusual resistance of hepatitis viruses to many procedures which inactivate common pathogens [8, 9] and the possibility of altering the usefulness of tissues when measures adequate to inactivate SH virus are applied present many problems Effective penetration of bone with its many canals and lacunae is difficult to attain by chemical sterilizing agents Since gamma rays are known to be powerful penetrating sources of energy effective in killing a wide range of microorganisms experiments were designed to determine the effect of radiation on bones from virus infected rats

For this study an infectious agent of small particle size was needed which reached a high concentration in the blood of infected hosts and yet was resistant to freezing and drying The agents used were the Novy virus isolated at the University of Michigan [30] and the Webster strain of eastern equine encephalomyelitis (EEE) virus Both viruses are approximately 30 to 40 mμ in diameter [13, 30] and reach a relatively high concentration in the hematopoietic organs of young rats Tibias from infected animals were removed immersed in distilled water and frozen After lyophilization one of the tibias was ground in a mortar suspended in saline and titrated for infectivity by intracerebral injection in mice The second tibia was irradiated and then titrated as indicated in Table 5 Results show that despite a tenfold concentration of virus in bone from Novy infected rats both viruses were inactivated at approximately 3 5 megarep The intensity of radiation required to inactivate viruses in bone is consistent with dosages described earlier for crude virus in suspension

Irradiated tibial segments transplanted into o rats resulted in gradual disappearance of the homografts with the formation of new bone None of

MICE INJECTED INTRACEREBRALLY
WITH LYOPHILIZED IRRADIATED BONE SUSPENSIONS
FROM VIRUS INFECTED RATS

VIRUS	NON IRRADIATED CONTROLS	MEGA rep* GAMMA RADIATION					
		10	20	30	35	40	45
NOVY $10^{-5.4}$ **	5/5	4/5	3/5	1/5	1/5	0/5	0/5
EEE $10^{-4.2}$	4/5	3/5	3/5	1/5	0/5	0/5	0/5

*ROENTGEN equivalent physicals in millions
†NUMERATOR number of deaths ; DENOMINATOR no. of mice used
** LD_{50} in bone after lyophilization
EEE Eastern Equine Encephalomyelitis virus

TABLE 5

the transplants became sclerotic or showed any evidence of sequestration Histologic examination of the graft sites at various intervals revealed gradual replacement of the transplant by newly formed bone without evidence of infection or foreign body reaction Since the beginning of these studies gamma irradiated bone has been used successfully in over 80 surgical procedures at the University of Michigan Hospital[31] More recent reports[7, 13] indicate that high intensity electrons are useful in sterilizing many different tissues intended for surgical transplant

Discussion

The results of these studies indicate that gamma radiation from cobalt^{60} is an effective method of inactivating certain animal viruses in vitro Smaller viruses apparently require a greater intensity of radiation for inactivation than do larger viruses Although it would have been desirable to determine the effect of gamma radiation on hepatitis viruses in blood and other biologicals the lack of a susceptible laboratory species for SH virus has precluded its use in these experiments Since a relationship between virus size and the dose of radiation required for its inactivation does exist SLE virus which is approximately the size of SH virus was substituted for the etiologic agents of viral hepatitis in some experiments while EEE and Novy viruses which produce a viremia in rats were substituted in others It should be emphasized that virus substitution was based only on size or the ability of the infectious agents to produce viremia with awareness of the possible differences in environmental conditions necessary for their respective inactivation Experiments in which SLE seeded plasma was used showed that irradiated plasma could be administered intravenously without untoward reactions in patients Electrophoretic changes seen in plasma indicate that as radiation increases the

albumin fraction decreases The reasons for these and other changes seen in the electrophoretic patterns of irradiated plasma are unknown

Although it is generally accepted that immune globulin is unlikely to play a role in the transmission of virus hepatitis globulin was used in these studies to determine quantitatively any changes which might have occurred in biological activity during irradiation The neutralizing capacity of poliomyelitis immune globulin was apparently unaffected when exposed to radiation in excess of levels required for inactivation of the viruses tested With prolonged exposure to radiation globulin appears to have been reduced in concentration as detected by paper electrophoresis and measured in the Spinco Analytrol No apparent change however was detected in the neutralizing capacity against Type I and Type II poliomyelitis viruses These findings suggest that the observed reduction in immune globulin may well have been in fractions which contained little or no antibody or that fractions which were destroyed contained no specific antibodies for the viruses tested

Recent advances in orthopedic and vascular surgery have greatly increased the use of tissue homografts in human subjects The possibility of passenger viruses must always be considered when tissues are transplanted from donors to recipients Because of the nature of homografts the spectrum of sterilizing agents that can be used is necessarily limited Sterilization must be accomplished in the case of bone grafts without altering its ability to stimulate new bone formation in the host Results using virus infected bone show that gamma radiation is effective in inactivating at least two animal viruses in lyophilized tibial segments Data on levels of radiation required to inactivate large concentrations of virus in rat bones are consistent with doses obtained for viruses in whole brain and in suspension More important however gamma irradiated bone segments do not lose their usefulness as grafts The incidence of successful homotransplantation of gamma irradiated bone in experimental animals and man has been remarkably high In most instances the transplants were replaced by newly formed bone without evidence of sclerosis sequestration or foreign body reaction

Despite the encouraging results obtained thus far the preliminary nature of this study is emphasized The data suggest that gamma radiation from cobalt[60] may prove to be an effective method in preventing the transmission of virus hepatitis under certain conditions

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*The Combined Effect of Thermal and Ionizing Radiation on Viruses**

ERNEST C POLLARD PH D

(New Haven Connecticut)

The problem of control of hepatitis virus like that of any other virus is fundamentally complicated Where it is possible to characterize the virus experiment with it and directly devise ways of inactivation sterilization production of attenuated strains and so on it is often unnecessary to consider the properties of viruses in general because particular methods may readily lead to a solution With hepatitis there is no such luxury although the very beautiful work described by Dr McLean and Dr Gard gives us real hope that the lean years are coming to an end Until they do until laboratory virus growth and assay becomes practical we have to look at what we know about viruses in general and try to use the slender evidence at hand to tell us what are the probable rewards of any one line of approach to virus control The work in our group at Yale has been directed quite resolutely at studying virus structure and properties and also at understanding the action of ionizing radiation and although we have done no work at all with hepatitis I shall make no further apology but proceed to discuss the two effects of heat and ionizing radiation on viruses

STRUCTURE AND FUNCTION OF VIRUSES

In the past few years real progress has been made in describing the structure and function of viruse This has been partly due to electron microscopy to X ray diffraction and to radioactive tracer methods but it has also been aided by the use of ionizing radiation to inactivate specific parts of the virus and measure their size and shape We have been employing this technique for a number of years and have been among the first to draw schematic pictures of viruses which are inferential in character but which must convey some idea of the various parts and what they do I propose to show four pictures of viruses obtained in this way

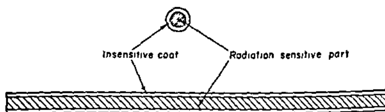
* The work described here was assisted partly by the Atomic Energy Commission and the John A Hartford Foundation

and then discuss in general what might be expected from virus inacti vation

The first and in many ways deceptively the simplest is tobacco mo saic virus It is a long rod with an infectious unit length of 3000 angstroms and seemingly has a rather thin insensitive coat with a part which is much more sensitive to radiation (Figure 1) X ray diffraction studies by Watson have established that the outer layer is a helical arrangement of protein molecules and recent work by Caspar and Rosalind Franklin have shown that the actual center is hollow In some way not yet estab lished the ribonucleic acid is arranged inside the protein helix

The second virus I would like to consider is T1 bacteriophage It is a DNA (desoxyribose nucleic acid) virus and it is just beginning to be realized that bacterial viruses for this very reason may not be typical In fact we may possibly face the classification of viruses as genetic and microsomal according to whether they contain nucleic acid as DNA or RNA (ribose nucleic acid) Such bacteriophages have an elaborate mor phology The vital part the DNA thread is somehow coiled inside the head of a protein structure which again is probably some kind of inter locked molecular casing The tail probably contains a unit responsible for bacterial killing and this unit may well be a section of DNA which is not in one long unit (Figure) Our evidence indicates that about 40 per cent of the virus s sensitive part is involved in this function At the end is an at tachment and entry unit which may bear some relation to the receptor destroying enzyme of influenza virus The virus attaches by its tail and upon incubation the entry unit opens a hole in the host and the remainder of the virus proceeds by a kind of linear Brownian movement to enter the bacterium The shell is left outside

A third virus is that of influenza (Figure 3) This figure represents deductions made from radiation studies by Dr Powell in collaboration



STRUCTURE OF TOBACCO MOSAIC VIRUS DEDUCED FROM RADIATION STUDIES

Figure 1 A schematic picture of tobacco mosaic virus deduced from radia tion studies by the author and Dr A F D mond

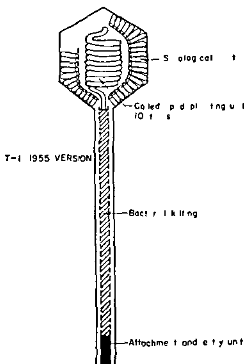


FIGURE 2 Schematic representation of T1 bacteriophage based on radiation studies A coiled up thread presumably of DNA resides inside a protein coat which has functional units as indicated

with Dr Jagger Dr Setlow and myself It is very much schematic and is intended more to represent the various sizes of the component parts Nevertheless it is of great interest that the beautiful section pictures taken by Dr Morgan and Dr Rose at Columbia show a set of concentric rings and also an over all size which agrees remarkably well with these findings

The fourth virus is that of Newcastle disease the NK variety The data are based on measurements recently made by Dr D Wilson and myself Again there is an insensitive coat in which there are apparently imbedded a set of about a dozen flat plate molecules These molecules are responsible for the hemolysin action There is also a set of rather smaller paired molecules which cause the agglutination of red cells Finally there is an inner sensitive region of nucleoprotein which carries the genetic part and also the interfering ability not yet studied (Figure 4)

The above pictures serve to fix one's impressions as to what a virus *is*

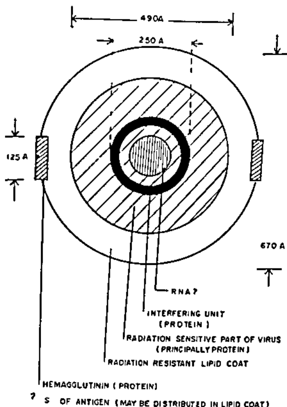


FIGURE 3 Schematic picture of influenza virus as drawn by Dr W F Powell Department of Pathology Yale

Among the things that it *does* is to enter (in whole or in part) the host cell to make its various component parts (or order them made by the cell) to assemble them and to escape In this connection I cannot help the parenthetical remark that since the lack of an experimental host is our major obstacle and that proof that we have discovered such a host may be hard the one significant aid in doing this is our possession of convalescent antiserum Such antiserum will specifically combine not only with completed virus particles but also with specific virus protein Such protein will be made *de novo* from amino acids It might well be that a study of labeled amino acids such as methionine and whether they become incorporated into a fraction which binds to convalescent antiserum might provide the best evidence that a host was at least tolerating virus

Virus Inactivation

The inactivation of a virus is due to the loss of function of one of its units In studies made on thermal inactivation dating back to quite early

work it was realized that the inactivation of a virus and the denaturation of a protein are rather similar Studies with ionizing radiation and with ultraviolet light have served to emphasize this broad similarity although these last two radiations actually diverge rather sharply from one another when a quantitative analysis is attempted Thus the inactivation of a virus requires statistically only a few ionizations while a rather large number of photons need to be absorbed for inactivation by ultraviolet light.

In Figure 5 a schematic picture of a virus is given to illustrate the problem of inactivation Three kinds of factors are shown the factor for entrance the antigenic surface and the long chain associated with infection The inactivation of any one of these functions will destroy the virus Both the entrance factor and the long chain are supposed to be unique while the antigenic surface is multiple To inactivate this last many molecular units have to be damaged while for either of the other functions a single protein or nucleoprotein has to be inactivated

When any kind of inactivation is observed it is the sum of all kinds of

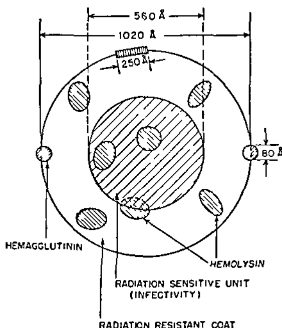


FIGURE 4 Representation of Newcastle disease virus based on radiation data taken by the author and Dr D Wilson There is definitely a radiation resistant coat with a sensitive (probably coiled up) RNA and protein center

damage which is seen unless specific experiment is carried out to separate the various factors

THERMAL INACTIVATION

Except in a few cases like tobacco mosaic virus (TMV) above 80° C and T5 phage above 60° C where *mechanical disruption* of the virus takes place the thermal inactivation of a virus fits with the picture of enzyme inactivation or protein denaturation To try to explain how this takes place consider Figure 6 For the moment please ignore the track of an ionizing particle and the positive ion and electron marks

The skeleton framework is a representation of the network of either a large protein molecule or a nucleic acid It is made up of strong covalent bonds and various grades of weaker bonds like hydrogen bonds or VanderWaals bonds It is assumed that the weaker bonds can often break but if the whole structure remains reasonably intact they will also re form Such temporary derangement has no deleterious effect However if two hydrogen bonds broke temporarily in a unit like the right hand chain the whole chain could bind up and a new set of hydrogen bonds might be formed which would bind the protein or nucleic acid in a new configuration Such a new configuration may well be permanent or nearly so and it may also destroy the biological action of the molecule breaking a genetic relationship or altering a specific relation to a substrate

This mechanism for heat inactivation was first to my knowledge pro posed by Augenstine

It fits very well with the Eyring theory According to this the prob

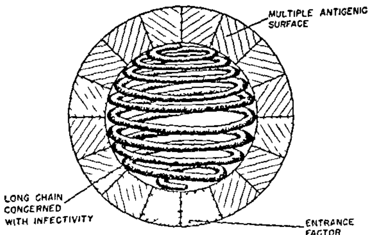


FIGURE 5 A generalized animal virus showing in token fashion three essential qualities the outer coat the entrance factor and the coiled genetic thread

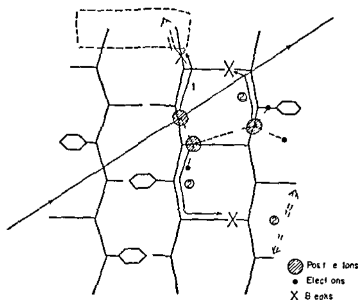


FIGURE 6 The skeleton of a protein or nucleic acid molecule shown to illustrate the process of inactivation. Bonds broken at X could weaken the whole integrity of the molecule so that it drifts apart and rejoins in a wrong way. If ionizing radiation causes weak spots which can migrate, then such thermal action is greatly aided.

ability that enough bonds will be open is given by the statistics of the activated state, which is the name Eyring gives to the point of no return on the way to the final denatured condition. These statistics are governed by two factors, the first the energy needed to break bonds, and the second the energy readjustment caused by the liberation of bound water.

At the risk of seeming obtruse and mathematical I intend to pursue these ideas for a moment because there is a chance that they may explain the seasonal variation of the incidence of hepatitis. To show briefly what happens, the two conditions of extreme wetness and complete dehydration of a macromolecule can be contrasted. In the first place the interstices of the network of Figure 6 are full of water, which is bound and not free to rotate. This water serves to make the bonds structurally stronger. Now as the right hand chain opens, it causes water to become free to start rotation and to diminish the energy of bonding. Thus when the inactivation starts, it goes with a rush.

In the dry state no water is present or, more correctly, much less water is present, and the bonds are all weaker. Even so, the transition to

damage which is seen unless specific experiment is carried out to separate the various factors

Thermal Inactivation

Except in a few cases like tobacco mosaic virus (TMV) above 80° C and T 5 phage above 60 C where mechanical disruption of the virus takes place the thermal inactivation of a virus fits with the picture of enzyme inactivation or protein denaturation To try to explain how this takes place consider Figure 6 For the moment please ignore the track of an ionizing particle and the positive ion and electron marks

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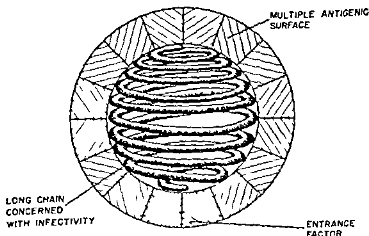


Figure 5 *A generalized animal virus sh ing in token fashion three essential qualities the outer coat the entrance factor and the c iled genetic threa l*

the range of temperature covered is from 80 to 150 C and the inactivation proceeds far more slowly as the temperature is raised This is because in the dry state $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ are both much lower Usually it is found that $\Delta S^{\ddagger}$ is zero or negative and $\Delta H^{\ddagger}$ is about four times less in the dry state

Seasonal Incidence of Hepatitis

The figures shown by Dr Paul and the fact that Dr Wood was able to heat his Staten Island cocktails to 56 C indicate that (1) the early summer months are low points in infection and (2) wet hepatitis can stand 56 C certainly for some minutes

If we are to use the thermal inactivation of virus while it is on the intestinal oral route to explain the seasonal incidence then clearly it must be able to survive in the wet state The Delhi outbreak proves this also On the other hand if the drying process were done rather slowly so that virus was kept in a moist but not wholly wet state it is conceivable that the lowering of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ would take place in such a way as to give a gradual widening of the temperature range of inactivation so as to cause the virus to be sensitive in a semiwet state Thus in the spring the ambient virus becomes less active In the summer the drying becomes rapid and the virus passes to a state where it is admittedly unstable but only slowly so In this way the virus remains intact until the wet winter season when it can make good headway in the population To complete this account it can also be added that the strength of the hydrogen bonds may well depend on pH As a drop evaporates the pH (unless it starts at 7) will move to a greater extreme This could be an added factor producing the same effect as formerly mentioned

Inactivation by Ionizing Radiation

A glance back at Figure 6 will give some insight into one form of inactivation by ionizing radiation I refer to the effect produced when an ionization is caused to occur in the particular molecular structure which can be inactivated This is often called the direct effect in contrast to an effect produced in a liquid medium and which can act chemically

When the ionizing particle traverses the molecular structure it can release an electron from occasional atoms There is no selection about this process it occurs about equally in any atom However an electron missing from an atom has two factors of importance to be considered The first is the fact that other nearby atoms have electrons which can rapidly exchange with any one atom It is in fact this exchange which is the basis of the covalent bond So the denudation of any one atom can rapidly be removed at the expense of a neighbor until the region of positive charge has traveled many times throughout the micromolecule The second fac

rotation will not occur and thus the energy of bonding while weak has no extra props so to speak and as a result the inactivation never proceeds with the same frenetic haste as when the release of water occurs All this appears very nicely in a formula due to Eyring It is as follows

$$k_1 = \frac{kT}{h} e^{-\frac{\Delta S^{\ddagger}}{R}} e^{-\frac{\Delta H^{\ddagger}}{RT}}$$

where k_1 is the reaction rate k is Boltzmann's constant T is the absolute temperature h is Planck's constant e is the base of natural logarithm $\Delta S^{\ddagger}$ is the entropy change to reach the activated state $\Delta H^{\ddagger}$ is the internal energy change to reach the activated state and R is the gas constant

In the wet state $\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$ are both large and inactivation rapidly increases with temperature as shown in a typical case in Figure 7 where the inactivation of wet T1 phage with temperature is shown For dry T1

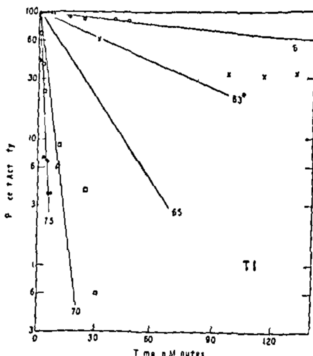
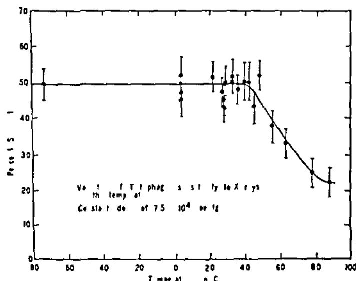


Figure 7 The thermal inactivation of T1 bacteriophage as observed by Marjorie Reaume and the author The whole measurable range of sensitivity is confined to 15 C This corresponds to a value of $\Delta H\pm$ of 95,000 calories per mole and $\Delta S\pm$ of 207 calories per mole per degree In the dry state $\Delta S\pm$ drops to zero and $\Delta H\pm$ to 27 5 0 The resulting range of temperature over which inactivation can be observed is far greater

Reaume and Powell shows that the cross section which measures the sensitivity of the invertase molecule to deuterons varies markedly with temperature as can be seen in Figure 8 Notice particularly that the cross section rises very greatly in the region where the inactivation of the invertase would just be beginning At the highest point given a few per cent inactivation due to temperature alone would be expected in the case of invertase The synergistic effect of radiation and heat is thus clearly seen

Turning to the effect on a virus Figure 9 which is taken from the work of the author and Adams shows the effect of heat on the amount of inactivation of T1 due to a single dose of X rays It can be seen that the sensitivity increases markedly and even so there is very little inactivation by heat alone at the point where the molecule is the most sensitive

It is therefore seen that it is perfectly possible under favorable circumstances a factor of 3 in increased sensitivity could be obtained by irradiating at a slightly higher temperature The preliminary results which have been obtained by Dr Murray on the variation of the inactivation of hepatitis virus with temperature would indicate that quite a low temperature might suffice for this purpose if indeed irradiation at say 40 degrees would suffice to increase the sensitivity to ionizing radiation it might



F ure 9 The relati e sen t ts of T1 bacteri ph ge t X ra a fun t n f t n perature At a temperature f 8 C here rel t ely little therm l ina ti at n take place the e tr ffe t f X rays d ble that n the ler c nd t on

tor is the great weakening of any bond when the shared electron is removed.

Thus after irradiation regions of weakness travel rapidly throughout the molecule, and if at the moment of passing a section is momentarily ready to move to a second form as we described above the radiation will greatly aid this process. Often the weakening is sufficient to cause radiation action alone to produce all the damage necessary to remove the biological function. Where this is the case the inactivation of the molecule so that it no longer fulfills its biological function will take place when the ionization has occurred anywhere within the structure. Such an all or nothing phenomenon is well known in radiobiology, but it is also possible that the molecule be large enough so that one ionization will not complete the inactivation. In particular in the case of a virus it is possible that radiation released in an unimportant part can put a little energy into a more important region but this may be insufficient to cause the inactivation of that region. Under those circumstances the existence of bonds already weakened by heat may be sufficient to cause the inactivation of the structure. To illustrate this we can consider the effect of ionizing radiation and heat on the enzyme invertase. Work done by the author

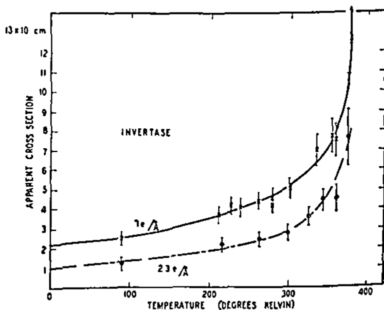


FIGURE 8 The relative sensitivity (plotted as an apparent cross section) of invertase which is held at different temperatures while being bombarded by deuterons. During the time of heating even at the highest temperatures no more than a few per cent of invertase is inactivated purely thermally.

GENERAL DISCUSSION

RALPH W BRAUER, PH D (San Francisco California) From the work of our friend Alexander in England we have had a fair amount of information on the oxygen effect of radiation sensitivity of various macromolecules I believe I did not hear any discussion of that in relation to the hepatitis virus. Since these effects are quite differential on different macromolecules, it seems to me that in modifying oxygen tensions from high to low in respect to Alexander s data this would be down in the subatmospheric pressure regions and it might be possible to enhance the differential effects that one would like to get without killing off one s proteins

ERNEST C. POLLARD PH D (New Haven Connecticut) I would like to comment on Dr Brauer s remarks Dr Alexander also has very nice independent evidence for this thermal effect which is quite good I usually have the effect of stopping any discussion when I speak and I should have been put on the program first It is the exponentials that are worrying people

I would like to open a little discussion on what is known about the thermal inactivation of the virus There are quite some contradictory factors coming in I might enumerate them

First of all as Dr Allen has observed in about 6 months at room temperature the virus is apparently inactivated Second is the observation that at 60 C. for ten hours the serum is apparently all right to use On the other hand the Willowbrook patients who were given Staten Island cocktails (and this had been prepared for some length of time at 56) behaved appropriately In other words the virus is not inactivated at 56 in one case whereas it is inactivated both at 60 and at room temperature

It seems to me that there is an inconsistency and perhaps someone might remark on it For example I would like to know how long the 56 exposure for the Staten Island cocktail took place That might be informative

ROBERT WARD M D (New York New York) That was 56 for half an hour Dr Pollard Also it may have been a different virus Perhaps it was a hepatitis virus rather than a serum virus

JOSEPH STOKES JR M D (Philadelphia Pennsylvania) I should be interested in asking Dr Pollard how much experience he has had with the length of incubation period after treatment by heat

In the hundreds of epidemics I have seen of ordinary epidemic hepatitis the length of incubation that Dr Ward and Dr Hoaglund have described

very well be possible to retain not only the albumins but the globulins in reasonable condition while at the same time removing the activity of the virus itself

Clearly further experimentation is required along these lines but it does seem to offer one of several favorable avenues of approach

Summary

Some schematic drawings of viruses based on radiation and electron microscopic studies have been given The multiple character of the inactivation of viruses has been stressed and consideration given to the effect of heat alone on the inactivation of viruses

The variation in sensitivity with humidity has been noted and it has been suggested that this may well be a basis for the seasonal variation of the incidence of infectious hepatitis

A theory of the effect of ionizing radiation and heat on virus inactivation has been outlined and it is suggested specifically that the irradiation of serum at temperatures a little bit above 40 degrees might give a considerably improved removal of the virus with very little damage to the proteins

million rad The unit rep has also been used and the rad and rep are very close together The rep refers to an energy absorption of about 83 ergs per gram and the former about 100 ergs per gram They are almost equivalent units

I have found Dr Pollard s explanations most interesting and of course the direction of his explanation was that if one elevated the temperature and took on the advantage of the thermal weaknesses to assist radiation destruction one might end up with a differential advantage in favor of destroying the virus At least the radiation dose required to destroy the virus might be reduced and this might be differentially advantageous Actually we are contrasting the destruction or inactivation of a small structure with the destruction of a large protein molecule and my impression would be that the combination of heat with radiation would in fact be disadvantageous

The differential action might well go the other way In fact I think perhaps one of the most important ways of preserving complex molecular structures that has been found in all studies on the action of radiation on protein molecules and organisms has been the reduction rather than the increase in temperature That is to say a threefold reduction of required dose by the use of an increase in temperature is not as influential in preserving the medium as let s say radiation at this maintained dose down to dry ice temperature

Of course this is only preliminarily borne out by experiment but I think there is a fair amount of evidence to suggest that in general this would be true.

Russell T Jordan M D (Duarte California) I think that in the consideration of inactivation by ionizing radiation one must always consider the purity of the preparation that is being tested I believe that is extremely important and even extremely minute amounts of extraneous protein can have a rather large difference in the doses required to inactivate certain particles

It is extremely important also to consider in certain instances the medium in which the bacteria were propagated We have some preliminary studies using spores in which the medium seems to have a significant bearing on the doses required for inactivation

Also I think when we consider the inactivation of any particle we must also bear in mind its size

Thomas Francis M D (Ann Arbor Michigan) Dr Pollard in your sketch of the universal virus on the outline you had the multiple antigenic factors Is there any evidence which you have that indicates that one of

is extremely unusual We have never seen this except in some specimens that have been obtained from plasma In ordinary outbreaks of hepatitis I have not seen an incubation period of this type and I wonder how many viruses have been treated at this temperature to determine the incubation period

Dr Pollard The answer as far as I know is that there is none Actually as far as I can tell this whole aspect of what happens to the virus due to humidity and temperature conditions has not been studied from the epidemiological viewpoint I am merely putting in my two bits that it might be worth looking at

John R Paul M D (New Haven Connecticut) I don t know whether Dr Havens is in the audience but he has had some experience which deals with Dr Stokes s question — at least in heating a strain of infective hepatitis virus A in his experimental work of some twelve years ago in which it received the same treatment as Dr Pollard referred to as the Willowbrook cocktail and there the incubation period was not shortened

I think the treatment was the same Perhaps Dr Ward will correct me if I am not absolutely accurate

I have been interested in this new type of approach for inactivating biological agents and I have been wondering to some extent how far the work with bacteria has gone This is the hardest type of approach to work with a virus but here are all these agents for destroying active infectious material and it would seem that one might start with that unless it is completely beside the point

John G Trump D Sc (Boston Massachusetts) I would be happy to make a brief remark on the effect of radiation on bacteria

Bacteria in fact have been more thoroughly studied than viruses and part of the reason for the interest in the study of bacteria has been because of the interest in both producing sterile pharmaceutical products and in the preservation of food

I think it is now quite well established that all types of bacteria can be destroyed and again each one has a well defined lethal dose required depending upon its concentration It is also dependent upon the medium It is relatively independent of the temperature of radiation and Dr Pollard s very fine curve which showed that from room temperature down to dry ice the sensitivity to radiation remained constant seems to be true for virtually all bacteria

The spore formers are the toughest to destroy and in very high concentration on the order of 1 or 10 million organisms per ml *Bacillus subtilis* for example — which is a tough one — might require about 3

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*Chemical Sterilization of Whole Blood and Plasma with Beta-Propiolactone**

GERALD A. LoGRIPPO, M.D. and CLARENCE E. RUPE, M.D.
(Detroit, Michigan)

In an attempt to satisfactorily sterilize whole blood and plasma, over 600 chemicals were evaluated for their virucidal activity in vitro. These agents can be divided into 3 general groups: (1) chemical agents, (2) physicochemical agents, and (3) combinations of these.[1, 2]

Since the hepatitis virus was not available in the laboratory as a test object, viruses were selected which produce viremia in animals, are resistant to inactivation, differ in chemical composition and are infective by a peripheral route. Two viruses meeting these requirements were the MM strain of mouse encephalomyocarditis (MM) and the eastern equine encephalomyelitis virus (EEE). A third virus, lymphocytic choriomeningitis (LCM), was selected because it is intimately associated with the erythrocyte and could be used to demonstrate the mechanism of drug action with certain compounds. The 3 different virus test objects used in these studies were: (I) 20 per cent virus infected tissue in 10 per cent serum saline solution reduced to 10 per cent virus suspension by addition of equal parts of drug solution, (II) 10 per cent virus infected tissue in 90 per cent ACD whole blood and 10 per cent virus infected tissue in 90 per cent ACD plasma, and (III) whole blood and plasma obtained from virus infected animals during the stage of viremia.

In the preliminary screening of chemicals the agent was given every opportunity to exhibit its virucidal action by using a virus test object (Number I listed above) containing only 10 per cent extraneous proteins when equal quantities of virus suspension and drug solutions were mixed. Although 23 agents were found completely effective against 1 of the viruses, only those agents which were totally effective against 2 or more viruses were selected for further study. In test objects II and III only 1 part of drug solution was mixed with 9 parts of virus infected plasma or whole blood. Under these conditions the virucide was required to demon

* Assisted by grants from the Atomic Energy Commission, Department of Defense, Department of the Army Chemical Corps, and the Research and Development Division, Office of the Surgeon General, Department of the Army.

these is more susceptible or can be selectively inactivated and others left behind or are they all very much of the same resistance or susceptibility

Dr Pollard The question is whether there is any difference in the sensitivity of the antigenic surface molecules I would very much like to answer that

The truth is that there are very few viruses for which these can be separated readily and not enough work has been done on those that can be We are planning to do some such work There is a difference in the sensitivity of the hemagglutinin and the hemolysin and we think these are surface factors

31

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strate its ability to inactivate all detectable virus in the presence of high concentrations of extraneous proteins

Of the 600 compounds tested in the preliminary screening with 10 per cent serum only 16 compounds demonstrated complete inactivation of or more viruses These are shown in Table 1 Of these 16 compounds only the last 5 were effective in 90 per cent plasma and out of the 5 only beta propiolactone (BPL) and sulfur mustard completely inactivated or more viruses in 90 per cent whole blood Unfortunately both of these agents produce complete hemolysis in whole blood at the minimal effective virucidal concentrations BPL was the drug of choice for plasma sterilization for the following reasons (1) It is water soluble (2) it is less hazardous to handle (3) it degrades spontaneously to relatively nontoxic substances (4) it is less deleterious to plasma proteins and erythrocytes at the virucidal concentrations and (5) there is a wider margin of safety between virucidal concentrations and toxicity levels for intravenous

TABLE 1

DEGREE OF VIRUCIDAL ACTIVITY OF EFFECTIVE AGENTS AGAINST 10 PER CENT FEL AND 10 PER CENT MM-VIRUS SUSPENSIONS

Incubation 37 C per 2 hrs

	Drug Concentration (mg per l) and Degree of Virus Inactivation *		
CHEMICAL AGENT	*10% Serum* (MDC) †	*90% Plasma* (MEVC) ‡	*90% Whole Bl* (MEVC)
Glycidol	50 000-C *	10 000-P	
Dimethyl amino-methyl 6-methoxy 4-chromanone HCl	10 000-C	10 000-P	
2 2 dimethyl ethyleneimine	25 000-C	25 000-P	
Ethyleneimine	2 500-C	2 500-Q	
N phenylethyleneimine	2 500-C	2 500-Q	
Ethyl acetylene dicarboxylate	5 000-C	5 000-P	
1 2 3 4-diepoxybutane	10 000-C	5 000-P	
Hydroquinone	25 000-C	25 000-P	
p-Quinone	2 000-C	2 000-P	
N-chloro-p-quinone	2 000-C	2 000 P	
Iodine in KI	2 500-C	2 500 P	
Butylene oxide	3[illegible] 500 C	32 500-C	32 500-P
Ethylene oxide	20 000-C	20 000 C	20 000-P
Paraformaldehyde	5 000 C	5 000-C	5 000 P
Sulfur mustard (propylene glycol)	3 000-C	2 000-C	2 000-C
Beta propiolactone	20 000-C	3 000-C	4 000-C

Q = Questionable P = Partial C = Complete
† Minimal drug concentration
‡ Minimal effective virucidal concentration

TABLE 2

DYES POSSESSING NO VIRUCIDAL ACTIVITY BY PHOTODYNAMIC ACTION AGAINST LLL, MM AND ECM VIRUSES SEEDED IN 90 PER CENT ACD PLASMA

Class	*Color Index Number*	*Name*	*Reaction*	*Maximum Absorption*
1 Thiazol	813	Titan Yellow	Acid	566 5
2 Anthroquinone	10 7	Alizarin	Acid	
3 Quinone imines		Kresylecht Violet (Cresyl Violet)	Basic	585 Natl An 630
	877	Brilliant Cresyl Blue	Basic	631 8 (579 5)
	825	Neutral Red	Basic (weak)	5[illegible]6
	841	Safranin D	Basic	530
	828	Azocarmine G	Acid	(540 5) 501 (467?)
4 Phenyl methanes	707	*Aniline Blue*	*Acid*	*600*
	657	Malachite Green (Oxalate)	Basic (weak)	616 (430)
		Rosaniline (HCl) (Magenta I)	Basic	54[illegible]
	680	*Methyl Violet (Mixture)*	*Basic*	*583*
	692	Acid Fuchsin (Mixture)	Acid	545
	677	Basic Fuchsin	Basic	539
	684	Methyl Green (Double green SF)	Basic	633 8
	706	Methyl Blue	Acid	607
5 Xanthenes		Atabrine		
		Mercurochrome 220		
	766	Fluorescein	Acid	490
	768	Eosin Y	Acid	516
	90	Acriflavin (Trypaflavin)	Basic	
6 Natural dyes	1180	Indigo Carmine	Acid	
	1246	Hematoxylin		
7 Miscellaneous dyes	370	*Congo Red*	*Acid*	497
	20	Chrysoidin Y	Basic	461
	477	Trypan Blue	Acid	
	248	Sudan III	Acid (weak)	641 (590)
	133	Janus Green	Basic	59[illegible] 7
	27	Orange C	Acid	485
	7	Picric Acid	Acid	360
	5	Naphthol Green B		

administration In addition BPL has a wide virucidal spectrum having inactivated all of the viruses against which it was tested (a total of 9 in all)

A different namely a physiochemical approach to virus inactivation was attempted by making use of the photodynamic action of dyes [8] Here virus inactivation is more specific since inactivation occurs only on those protein particles on which the dye binds An advantage to this approach is the small amount of dye required for sterilization in comparison to

strate its ability to inactivate all detectable virus in the presence of high concentrations of extraneous proteins

Of the 600 compounds tested in the preliminary screening with 10 per cent serum only 16 compounds demonstrated complete inactivation of 2 or more viruses These are shown in Table 1 Of these 16 compounds only the last 5 were effective in 90 per cent plasma and out of the 5 only beta propiolactone (BPL) and sulfur mustard completely inactivated or more viruses in 90 per cent whole blood Unfortunately both of these agents produce complete hemolysis in whole blood at the minimal effective virucidal concentrations BPL was the drug of choice for plasma steriliza tion for the following reasons (1) It is water soluble (2) it is less haz ardous to handle (3) it degrades spontaneously to relatively nontoxic substances (4) it is less deleterious to plasma proteins and erythrocytes at the virucidal concentrations and (5) there is a wider margin of safety between virucidal concentrations and toxicity levels for intravenous

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Dimethyl amino-methyl 6 methoxy 4 chromanone HCl	10 000-C	10 000-P	
2 2 dimethyl ethyleneimine	25 000-C	25 000-P	
Ethyleneimine	2 500-C	2 500-Q	
N phenylethyleneimine	2 500-C	2 500-Q	
Ethyl acetylene dicarboxylate	5 000-C	5 000-P	
1 2 3 4-diepoxybutane	10 000-C	5 000-P	
Hydroquinone	25 000-C	25 000-P	
p-Quinone	2 000-C	2 000-P	
N chloro-p-quinone	2 000-C	2 000-P	
Iodine in KI	2 500-C	2 500 P	
Butylene oxide	32 500 C	32 500-C	32 500-P
Ethylene oxide	20 000 C	20 000 C	20 000-P
Paraformaldehyde	5 000-C	5 000 C	5 000 P
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TABLE 2

DYES POSSESSING NO VIRUCIDAL ACTIVITY BY PHOTODYNAMIC ACTION AGAINST LEE, MM AND ECM VIRUSES SEEDED IN 90 PER CENT ACD PLASMA

Class	*Color Index Number*	*Name*	*Reaction*	*Maximum Absorption*
1 Thiazol	813	Titan Yellow	Acid	566 5
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	8	Brilliant Cresyl Blue	Basic	631 8 (5 9 5)
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		Mercurochrome 2 0		
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	768	Eosin Y	Acid	516
	790	Acriflavin (Trypaflavin)	Basic	
6 Natural dyes	1180	Indigo Carmine	Acid	
	1246	Hematoxylin		
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TABLE 3

DYES POSSESSING VIRUCIDAL ACTIVITY BY PHOTODYNAMIC ACTION*

Color Index Number	*Name*	*Maximum Absorption*	*Degree of Virus Inactivation†*		
			MM	*LCM*	*EEE*
922	Methylene Blue Polychrome	667	N	C	C
922	Methylene Blue Chloride	667	N	C	C
922	Methylene Blue Medicinal	667	N	C	C
923	Azure II	65[illegible]			
922	Mix Giemsa and Methylene Blue	667	N	C	C
923	Giemsa Mix Azure B predominating	652	N	C	C
923	Methylene Azure A	638	N	C	C
920	Thionin (Blue)	602	N	C	C
925	Toluidine Blue O	635	N	C	C

* Routinely screened at dye concentration of 10 mg per l against 10 per cent virus suspensions in 90 per cent ACD plasma
† N = None C = Complete

compounds which utilize chemical action only The procedure for the routine testing of the photodynamic action of dyes against viruses seeded in plasma follows A 5 ml volume of dye virus plasma mixture is placed in a 600 ml round bottle and slowly rotated for 1 hour on a mechanical rotator at the rate of 1 rpm while being irradiated with a 100 watt tungsten filament bulb at 1 foot distance The rotator is kept in an ice bath which maintains the plasma mixture at 4 to 9 C during irradiation All dyes were tested at 10 mg per liter drug concentration against 10 per cent virus seeded in 90 per cent acid citrate dextrose (ACD) plasma because higher concentrations usually interfere with light absorption and photo reaction

Thirty eight dyes were tested for their ability to inactivate 3 viruses (EEE MM and LCM) These are listed in Table 2 The dyes selected represent 7 different classes and were chosen because they have demonstrated photodynamic activity in biological systems other than virus seeded plasma These agents also represent fat soluble fluorescent acid neutral and basic varieties of dyes The 31 agents on this table demonstrated no virucidal activity against the 3 test viruses used However a group of thiazine dyes under the class of quinoneimines (listed in Table 3) were found to be very effective virucides against the LCM and EEE viruses although they showed *no* virucidal action against the MM virus

Since the 8 dyes are either derivatives or mixtures of methylene blue or toluidine blue these 2 dyes were chosen for further studies Table 4

TABLE 4

PHOTODYNAMIC ACTION OF METHYLENE BLUE

EEE Virus Seeded in Human ACD Blood

100-Watt Tungsten Filament Light bulb	*Time (min)*	*Virus Titer (LD₅₀)* *
Methylene blue (10 mg per liter)		
Plasma		
1 Virus control	30	7 6
2 Dye	15	0 0
3 Dye	30	0 0
Whole blood		
4 Virus control	60	7 2
5 Dye	30	2 6
6 Dye	60	2 2 (hemolysis)

* Negative log of dilution

demonstrates the virucidal activity of methylene blue tested against EEE virus seeded in human ACD blood Methylene blue will reduce the virus activity in plasma to nondetectable quantities in 15 minutes whereas in whole blood it has not completely inactivated the virus at the end of 60 minutes By this time there is marked hemolysis of the erythrocytes Toluidine blue on the other hand is equally effective in plasma and will inactivate the virus completely in whole blood in 30 minutes (Table 5) This is true of *naturally infected* as well as *artificially seeded* whole blood In this system the margin of safety between sterilization and hemolysis is too narrow Although sterilization is obtained in 30 minutes hemolysis begins in 30 minutes and is very marked in 60

Since toluidine blue proved less deleterious to the erythrocytes and effective in naturally infected blood it was also tested against the LCM virus which is known to be intimately associated with the erythrocytes when the blood is taken from infected animals Table 6 demonstrates the effect of toluidine blue on plasma washed blood cells and whole blood obtained from infected guinea pigs during the height of viremia In plasma the virus was reduced to nondetectable quantities in [illegible] minutes whereas with washed blood cells and whole blood 60 minutes does not reduce the virus significantly and 240 minutes is required to reduce the virus completely

The photodynamic dyes are relatively nontoxic at the drug concentrations required and the procedure is simple and rapid However they have been found effective against only 2 out of 5 viruses tested In addition sterilization is practical only with plasma and even here the treated plasma is turbid and the fibrinogen tends to clot on storage Work with the photodynamic dyes is being continued particularly in the sterilization of

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Although the combination of BPL and sulfur mustard is additive the necessary concentrations for complete inactivation of the MM virus offer no advantage as toxic levels of sulfur mustard are still required The combination of BPL and ultraviolet irradiation has been most promising and appears to be one of the answers to the sterilization of plasma [1 4 6 7]

Unfortunately the present method for sterilization of whole blood is not as satisfactory as that for plasma since the virucidal concentrations produce hemolysis Figure 1 illustrates this problem Four of the most promising virucides are shown here with the concentrations required to inactivate 3 different viruses seeded in whole blood The arrows point out the concentrations at which hemolysis begins as compared to the virucidal concentrations for the respective viruses The wide variations between the virucidal and hemolytic concentrations of each chemical agent for each virus make it clear that no predictions can be made concerning the hepatitis agent until it itself is studied

Although BPL is included in Figure 1 some of its unique properties suggested that it might be possible with this drug to avoid hemolysis and achieve sterilization if the erythrocytes were separated from the plasma and the two sterilized independently There are 5 properties of BPL which suggested a solution

(1) *The concentration of BPL required for virus sterilization is lowered when the total protein content is decreased* This is illustrated in Figure 2

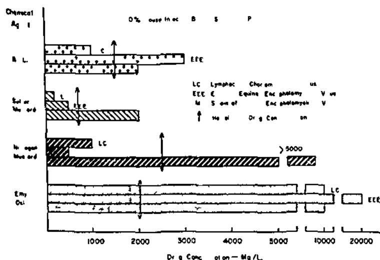


FIGURE 1 The effect of 4 chemical agents upon 3 different viruses

TABLE 5

PHOTODYNAMIC ACTION OF TOLUIDINE BLUE

EEE Virus Seeded in Human ACD Blood

100-Watt Tungsten Filament Light Bulb	*Time (min)*	*Virus Titer (LD_{50})* *
Toluidine Blue (10 mg per liter)		
Plasma		
1 Virus control	30	7.5
2 Dye	15	0.0
3 Dye	30	0.0
Whole blood		
4 Virus control	30	7.2
5 Dye	30	0.0

* Negative log of dilution

TABLE 6

PHOTODYNAMIC ACTION OF TOLUIDINE BLUE

LCM Virus from Infected Guinea Pigs

100 Watt Tungsten Filament Light Bulb	*Time (min)*	*Virus Titer (LD_{50})* *
Toluidine blue (10 mg per liter)		
1 Plasma		
(a) Virus control	20	4.3
(b) Dye	20	0.0
2 Washed Blood Cells in N Saline		
(a) Virus control	60	2.8
(b) Dye	60	0.9 (Hemolysis)
3 Whole Blood		
(a) Virus control	40	4.6
(b) Dye	60	1.[illegible] (Hemolysis)
(c) Dye	120	0.6 (Hemolysis)
(d) Dye	240	0.0 (Hemolysis)

Negative log of dilution

whole blood However of the chemical and physiochemical agents tested to date BPL continues to be the drug of choice

In an attempt to enhance virucidal activity of BPL below hemolyzing concentrations and thus make it suitable for whole blood sterilization combinations of BPL with a number of agents were tested These were nitrogen mustard sulfur mustard ethylene oxide ethyleneimine N chloro p quinonimine ultraviolet irradiation and toluidine blue with photochemical action The virus used in the combination tests was the one most resistant to the respective agent With the exception of sulfur mustard and ultraviolet irradiation no additive or synergistic effect was noted

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Although BPL is included in Figure 1 some of its unique properties suggested that it might be possible with this drug to avoid hemolysis and achieve sterilization if the erythrocytes were separated from the plasma and the two sterilized independently There are 5 properties of BPL which suggested a solution

(1) *The concentration of BPL required for virus sterilization is lowered when the total protein content is decreased* This is illustrated in Figure 2

VIRUCIDAL DRUG CONCENTRATIONS IN ACD WHOLE BLOOD

1000 2000 3000 4000 5000 10000 20000

Drug Concentration—Mg/L.

FIGURE 1 The effect of 4 chemical agents upon 3 different viruses

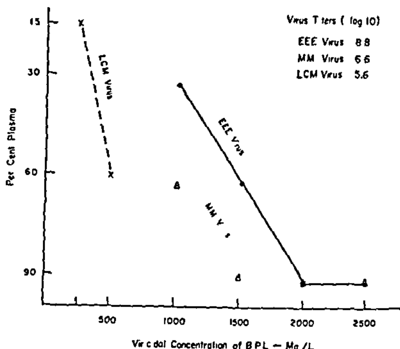


FIGURE 2 The effect of varying protein concentrations on the viricidal action of beta propiolactone (BPL) Each point on the curve indicates the drug concentration at which the virus titer is reduced to nondetectable quantities

where the per cent plasma is plotted against the drug concentration required for total inactivation of LCM, MM and EEE viruses When the virus titer is kept constant and the total protein content varied the BPL concentration required for complete virus inactivation varies directly with the amount of protein present i e the lower the protein content the less the amount of drug required

(2) Figure 3 demonstrates the second property i e *the concentration of BPL required for virus sterilization is lowered when the virus titer is diminished* Here the plasma protein content was kept constant and the virus titer varied by tenfold dilutions from 10^{-1} to 10^{-} This point can be best illustrated at the drug concentration of 1000 mg per liter where a virus titer of 10^{-3} shows complete inaction of 4 logs whereas at 10^{-} and 10^{-1} (which are 5 and 6 virus logs respectively) the virus is not completely inactivated In other words the lower the virus titer the lower the drug concentration required to reduce the virus to nondetectable quantities

(3) *The hydrolysis of BPL as measured by total titratable acid lags behind the rate of virus inactivation* (Figure 4) The virus titer is plotted

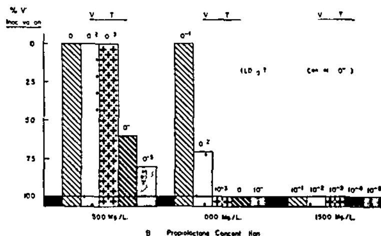


FIGURE 3 The effect of beta propiolactone on varying virus concentrations when the plasma proteins are kept constant

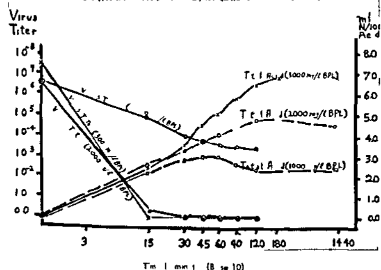


FIGURE 4 Complete virus inactivation is accomplished before the half life of beta propiolactone 28 to 32 minutes at 37 C (not indicated on the figure)

against the time required for inactivation while the total acidity is indicated on the right. Three concentrations of BPL are shown here (1000, 000 and 3000 mg per liter). Complete virus inactivation is obtained before the half life of the drug (8 to 3 minutes at 37 C) when adequate concentrations of BPL are used. Since the viruses are inactivated before the bulk of the acid is formed the erythrocytes could be removed at approximately the half life of BPL without interfering with sterilization and avoiding the deleterious effects of excess acid.

(4) This is possible because *BPL inactivated viruses are irreversible* (Figure 5). The solid line marked with arrows indicates the rate of MM virus inactivation while the lighter solid line shows the kinetic rate of hydrolysis of BPL. Sodium thiosulfate will combine with BPL mole per mole and stop virus inactivation. At the time intervals indicated by the arrows aliquots of the plasma virus drug mixture were removed and sufficient thiosulfate added to combine completely with the total amount of BPL present. These aliquots were then assayed for virus activity at 3 and 4 hours indicated by the dotted lines. The neutralization of BPL by thiosulfate is clearly shown in aliquots 1 and . The irreversibility of virus inactivation is demonstrated by the fact that in no aliquot did the virus titer rise significantly. What is more important from the standpoint of sterilization is the fact that after the quarter life of BPL the addition

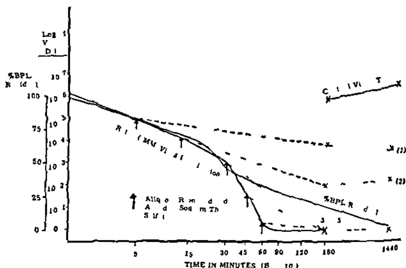
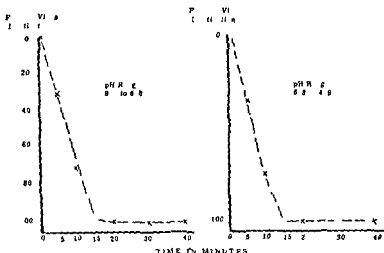


FIGURE 5. The irreversibility is demonstrated by the effect of sodium thiosulfate, a neutralizing agent, upon the virucidal action of beta propiolactone.



Fi ure 6 The virucidal action of beta propiolacton is independent of pH values

of sodium thiosulfate does not dissociate the bound BPL nor prevent sterilization as is demonstrated in aliquot curves 3 4 and 5

(5) The final property of BPL which we need to consider is that the *virucidal effect of BPL is independent of pH levels* Figure 6 demonstrates this property It can be readily seen that regardless of whether BPL reacts through a pH range of 9 2 to 6 8 or 6 8 to 4 9 there is no significant change in the rate of virus inactivation The pH of the medium can thus be adjusted to that which is least deleterious to the erythrocytes

One of the problems in using BPL is the acid products of hydrolysis The standard ACD solution used in the collection of blood is also acidic Dr Strumia s citrate dextrose lactose (CDL) solution which is alkaline not only proved to be a better reacting medium for BPL but also increased the osmotic resistance of the treated erythrocytes and substantially lowered the per cent hemolysis on 21 days of storage (Figure 7)

However even with the use of CDL solution 500 mg per liter of the drug cannot be exceeded without producing hemolysis This concentration is still not sufficient for the sterilization of whole blood By taking advantage of the properties of BPL and by treating the erythrocytes separately a sterilizing concentration (i e 4000 mg per liter of BPL) can be used without producing hemolysis if the treatment is conducted at



FIGURE In vitro effect of beta pr p lactone (500 mg per liter) on hemolysis.

4 C. and the erythrocytes removed before the half life of the drug The application of these principles is demonstrated in Figure 8 It can be seen that, when equal volumes of packed erythrocytes and BPL at a concentration of 8000 mg per liter are mixed, the drug concentration is reduced to 4000 mg per liter the plasma proteins are decreased and the virus titer diminished This drug concentration is required if the virus activity is to be reduced to zero before the half life of BPL which is 16 hours at 4 C. By removing the erythrocytes by the half life and resuspending them in an equal volume of CDL solution the drug concentration is further reduced to 000 mg per liter which is below the hemolytic concentration

Having treated the erythrocytes with virucidal concentrations of BPL and having obtained satisfactory in vitro results, it remained to extend the work to in vivo survival studies in man

In the preparation of the erythrocytes for transfusion, 1 volume of packed cells (50 to 300 ml) was combined with 1 volume of CDL solution containing 8000 mg per liter BPL This mixture was maintained at 4 C. for 8 hours the cells were then sedimented the supernatant fluid removed and equal volumes of CDL solution again added The mixture was then allowed to remain at 4 C. for 48 hours in order to obtain hydrolysis of residual BPL Just before transfusion the supernatant fluid was drawn off the cells brought to room temperature and the packed erythrocytes (approximately 50 ml) administered to the recipient.

The results are shown in Figure 9 The Ashby count method for in vivo survival of erythrocytes was used These determinations were made by Dr John Rebuck Division of Hematology Although of the 4 trans-

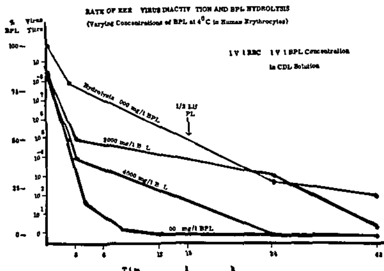
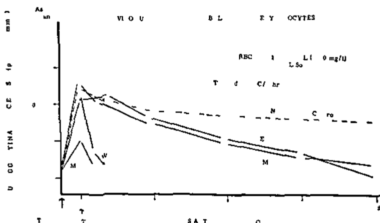


FIGURE 8 The sterilizing concentrations of beta prop olactone at 4 C and the concentration required to obt n sterilization before the half life of the drug



F GURE 9 The results of 4 tran fusions made vitl beta pr piol ctone treated erythrocytes

fusions show rapid disappearance of the cells in 24 to 48 hours the remaining show fair survival rates up to 14 days in vivo The poor results in recipient W P could not be re evaluated with an untreated transfusion as he expired 3 weeks later with an extensive carcinoma of long duration Recipient M B was a case of renal tuberculosis and the initial count showed rapid disappearance without a satisfactory transfusion peak A total of 4 transfusions is too few from which to draw any conclusions at this time More clinical data must be accumulated using the principle of treating erythrocytes with BPL and removal before the half life

Summary

After testing a large number of agents BPL (beta propiolactone) was shown to be the most promising virucide for chemical sterilization of biological material at the present time[8, 9, 10, 11, 12, 13] It has inactivated the 9 laboratory viruses thus far used as test objects and it is highly effective as a virucide in the presence of large amounts of extraneous proteins BPL can be used to sterilize plasma but the combination of the drug with ultraviolet irradiation is even more promising Whole blood cannot be satisfactorily treated with BPL but by taking advantage of some of its properties and by treating the erythrocytes separately it was possible to use virucidal concentrations of BPL without producing significant hemolysis

The in vivo survival studies though not as encouraging warrant continued clinical evaluation The fact that clinical trial is now possible affords the means of determining the efficacy of BPL against the hepatitis virus as it exists in blood collected for clinical use

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*The Toxicology of Beta-Propiolactone**

ANNETTA R. KELLY, Ph.D., FRANK W. HARTMAN, M.D.
and CLARENCE E. RUPE, M.D.
(Detroit, Michigan)

The previous speaker has discussed certain characteristics of the biological activity of beta propiolactone (BPL) which make it an intriguing and promising virucide. Its potentialities as a sterilizing agent for blood plasma, arterial homografts, bone transplants, vaccines and foods have necessitated a critical toxicity evaluation of the compound which is still in progress. This study has disclosed certain characteristics of BPL with respect to its aqueous reactions and the properties of its end products which combine to make it almost ideally suited toxicologically as a sterilizing agent for biologicals.

Chemistry of Beta Propiolactone

BPL is a stable liquid which reacts readily with hydroxyl,[1] carboxyl,[2] sulfhydryl,[3] amino[4] and phenolic groups,[5] all of which are associated with proteins. It hydrolyzes rapidly and completely in aqueous solutions and disappears much more rapidly in plasma than in water (Figure 1).

The chemistry of BPL involves ring opening at the alkyl or acyl oxygen bond (Figure 2). Opening at the alkyl bond generally involves ionic reactions which occur most readily in water and result in the formation of beta substituted propionic acids (Figure 2, Reaction 1).[6, 7] In reactions with 0.9 per cent sodium chloride approximately 3 per cent of the added BPL is converted to β chloropropionic acid. In plasma, smaller amounts are formed, amounting to only 2 to 10 per cent of the total added.

Opening of the ring at the acyl oxygen bond produces derivatives of hydracrylic acid (Figure 2, Reaction 2).[7, 8] At least 70 per cent of the initial BPL added to plasma can be accounted for by the formation of hydracrylic acid.

Although pure BPL is stable for relatively long periods, it is readily polymerized when catalyzed by acids, bases and certain salts.[8] In an aqueous medium the polymer molecules formed contain beta hydroxy

* This investigation was assisted by grants from the Atomic Energy Commission, the Veterans Administration and the Research and Development Division, Department of the Army.

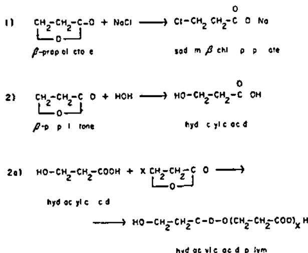


FIGURE 2 Reactions of beta propiolactone during hydrolysis in aqueous solutions

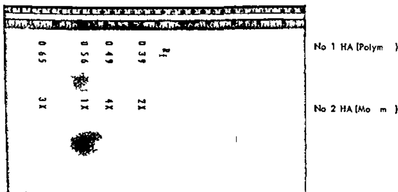


FIGURE 3 Reproduction of a chromatogram demonstrating separation of 4 polymers from a 58 per cent aqueous solution of hydracrylic acid

The extent to which polymer formation occurs was found to be directly related to the concentration of BPL undergoing hydrolysis (Figure 4) Only solutions more dilute than 1 Gm per cent (where one mole of BPL is reacting with more than 390 moles of water) are essentially free of polymers Since the highest BPL concentration employed in sterilizing plasma is only 0 6 per cent it is unlikely that hydracrylic acid polymers form to any significant extent in such plasma

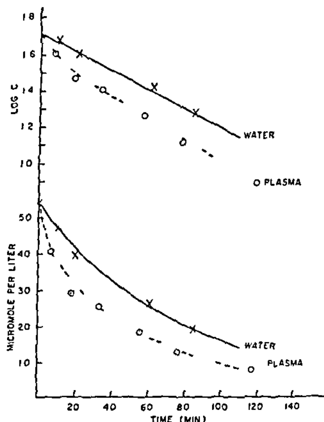


Figure 1 Hydrolysis rates of beta propiolactone (4000 mg per liter) in water and plasma at 37 C

end groups exclusively The first step appears to be the formation of hydracrylic acid (Figure 2 Reaction 2) which then reacts with another molecule of BPL to form a dimer regenerating the carboxyl end group *Continuation of this process produces higher molecular weight polymers* (Figure 2 Reaction A)

Detection isolation and quantitation of hydracrylic acid polymers in *solution was accomplished by chromatographic and chemical techniques*[9] The chromatogram reproduced in Figure 3 shows the monomer and three polymers isolated from a solution of hydracrylic acid titrating 58 Gm per cent *The monomer is the spot labeled 1X and its concentration wa* found to be as titrated However the actual total hydracrylic acid con centration when polymers were included was considerably higher — *75 Gm per cent The relative R_fs of the polymers and the probable* identification of dimer trimer and tetramer are indicated in Figure 3

I THE ACUTE TOXICITY OF BPL AND ITS DEGRADATION PRODUCTS

Undegraded BPL The comparative species toxicity of undegraded BPL is summarized in Figure 5 Intravenous LD_{50}s range from 345 mg per kg in the mouse [10-1] to 90 mg per kg in the dog [11 12]

Degradation products Hydrolysis of BPL produces a marked reduction in toxicity The intravenous LD_{50}s of the BPL degradation products show little species variation (Figure 5) ranging from 1 7 to 1 9 Gm per kg for sodium β-chloropropionate and from 2 0 to 2 4 Gm per kg for sodium hydracrylate the major degradation product [10-1]

The acute toxicities of the end products are affected by the state of hydration of the animal forced hydration allowing animals to tolerate LD_{50} and dehydration causing a significant increase in toxicity

The degradation products are only one half as toxic orally as intravenously their LD_{50}s being approximately 5 Gm per kg (Table 1) Hydracrylic acid is closely related structurally to the natural metabolite lactic acid differing only in the position of the hydroxyl group (Table 1) It is interesting to note that the LD_{50}s of the end products and lactic acid are quite comparable (Table 1)

Single near lethal intravenous doses of both degradation products pro

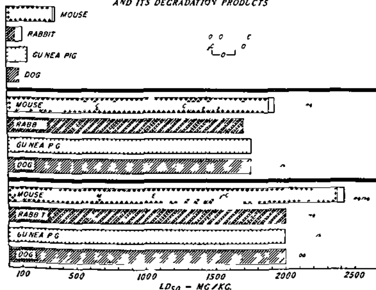


FIGURE 5

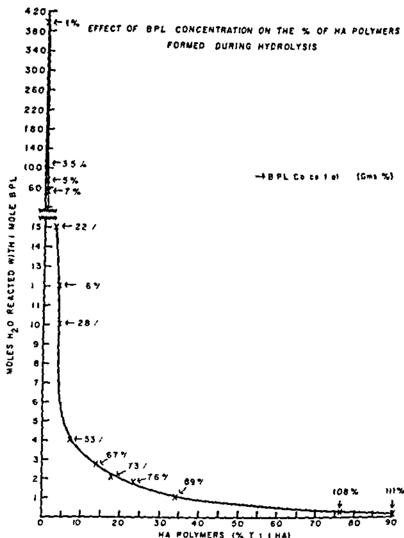


FIGURE 4

TOXICOLOGY OF BETA PROPIOLACTONE

The first phase of the toxicity evaluation of BPL was concerned with assessing the acute and chronic toxicities of the undegraded compound and its end products in four animal species The degradation products were studied both by administering the pure compounds and by observing the effects of massive chronic infusions of treated plasma

BPL (aqueous solution) Chronic intravenous administration of undegraded BPL produces marked cumulative toxicity in all animal species. Weight loss and necrosis of the kidney tubules and liver are the major findings[13, 14]

Degradation products (aqueous solutions) The degradation products show little tendency toward cumulation in any species. The only cumulative effects observed have concerned the platelets of the mouse and rabbit[1] The lower half of Figure 6 illustrates that large *single* equimolar doses of hydracrylic and β chloropropionic acids are each capable of producing an over all decrease in rabbit platelet count even when counts are corrected for blood volume changes. Hydracrylic acid is the more potent in this respect. However differences between the two compounds appear with multiple intravenous injections of the same doses (Figure 7). Studies of platelet morphology with the electron microscope indicate that β chloropropionate causes a rapid disintegration and disappearance of the aging platelet generation and although new cells are entering the circulation there is a small steady decline in count after each injection if the compound is being administered daily. The initial injections of hydra

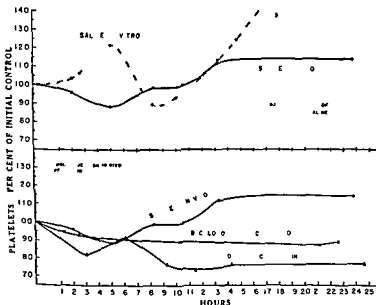


FIGURE 6 Comparison in the rabbit of effects on platelet counts produced by large single equimolar doses of each of the two degradation products

TABLE 1

COMPARISON OF IV AND ORAL TOXICITY OF BPL DEGRADATION PRODUCTS

Compound	Mouse LD_{50} Oral—mg/kg	Mouse LD_{50} IV—mg/kg
$CH_2Cl—CH_2—COOH$ β-chloropropionic acid (BCPA)	4850 ± 117	1900 ± 20
$CH_2OH—CH_2—COOH$ β-hydroxypropionic acid (HA)	5250 ± 338	2400 ± 78
$CH_3—CHOH—COOH$ α hydroxypropionic acid (LACTIC)	3020–4610*	2200

* Range of literature figures

duce changes which appear to be due primarily to the hypertonicity of the injected solutions. Diuresis, thirst, marked fluid and electrolyte disturbances, and electrocardiogram changes associated with hypervolemia are observed acutely. In the dog and rabbit, transient depression of the numbers of circulating lymphocytes and monocytes are observed, with a compensatory increase in neutrophiles and a slightly elevated total count. With lethal doses of β chloropropionic acid, disintegration of lymph node germinal centers has been observed if the animal survives at least 18 hours.[13]

II. THE CHRONIC TOXICITY OF BPL AND ITS DEGRADATION PRODUCTS

Table 2 summarizes the procedures and tests upon which assessments of the chronic animal and human toxicity of the degradation products and treated plasma have been based.

TABLE 2

LABORATORY TESTS FORMING BASIS OF CHRONIC TOXICITY EVALUATION

Test	Animal	Human	Test	Animal	Human
Complete hemogram	x	x	Thymol turbidity flocc	x	x
Platelet count	x	x	A/G	x	x
Sedimentation rate	x		*Cephalin cholesterol* flocc		x
Hematocrit	x		Blood urea nitrogen	x	
Bleeding time	x		NPN		x
Clotting time	x		Urea clearance	x	x
Clot retraction	x		Blood sugar	x	
EKG	x	x	Serum Na^+ K^+ Cl^-	x	
Body weight	x		Urine Na^+ K^+ Cl^-	x	
Food intake	x		Urine *p*H	x	
Urinalysis	x	x			
Tissue pathology at autopsy or sacrifice	x	x			

as per cent of the diet doses of 4 Gm per Kg or 75 per cent of the oral LD_{50} were ingested daily for nine months without significant toxicity

Degradation products (administered to animals in BPL treated plasma) Because of the probable formation of protein addition products which could not be studied individually the final and most crucial test of toxicity came from the chronic administration of BPL treated plasma in various animal species [10–1 14–16] and man [11 1 14 16] As far as platelet toxicity is concerned the major effects observed are due to hydracrylate since it is present in plasma at a level approximately 30 times that of the chloro derivative

A summary of some of the massive dosages of BPL treated plasma which have been administered to animals (Table 3) and man (Table 4 and Table 5) without encountering toxicity should promote a better apprecia tion of the small amounts of breakdown products present and allow a more accurate estimation of the potential toxicity

It should be noted (Table 3) that even when mice received in 9 days a volume of plasma (treated at 6 0 Gm per liter) which would be equiva lent on a weight basis to 38 l in the human the dosage of sodium β chloropropionate administered was only 65 mg per Kg this amount is equal to only 3 per cent of the LD_{50} of the compound Although an LD_{50} of hydracrylate was administered in this plasma over a 9-day period no evidence of toxicity was encountered

Rabbits received in 9 days the human equivalent of 16 liters of plasma treated with 4 0 Gm per liter of BPL The dosage of the chloro deriva tive was negligible (Table 3) and the hydracrylate amounted to only 70 mg per Kg per day

Full grown dogs received daily for 20 days the human equivalent of 500 ml of homologous plasma (treated with 4 Gm per liter of BPL) The amounts of degradation products administered were relatively small (Table 3)

Litter mate puppies received the human equivalent of 250 ml of treated homologous plasma per day from 3 to 9 months of age without detectable toxicity (Table 3) Slopes of the growth curves of treated and control animals were identical (Figure 8)

Degradation products administered to man in BPL treated plasma As soon as animal toxicity data indicated an adequate margin of safety clinical administration of BPL treated plasma was initiated For the past five and one half years the toxicologic evaluation of the degradation products in man has been in progress [11 1 14 16] In the absence of knowl edge of the LD_{100} of BPL for the virus of serum hepatitis it has been necessary to investigate the human toxicity over a wide range of treatment

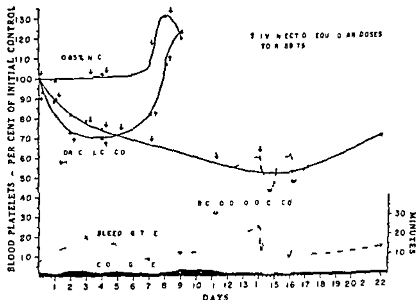


FIGURE [illegible]. Comparison in the rabbit of the effects on platelet counts produced by *multiple* daily equimolar doses of the two degradation products

crylate also cause disintegration and removal of old cells but soon each successive injection appears to have less effect on platelet numbers because the cumulative effects are being masked by simultaneous platelet fragmentation. Finally if injections continue some rabbits show massive platelet disintegration accompanied by an anaphylactic type response presumably caused by the massive release of histamine from the disintegrating platelets. Neither compound significantly affects bleeding time, clotting time or clot retraction because platelet numbers remain adequate.

β chloropropionate doses equivalent to 75 per cent of the LD$_{50}$ have been administered to mice daily for 38 days without effect on platelet numbers. However the role of the spleen in removing injured cells can be demonstrated in that splenectomized mice on the same dosage schedule showed a significant increase over control platelet counts and over the counts of splenectomized mice receiving saline. Doses of β chloropropionate below one fifth of the LD$_{50}$ have no demonstrable platelet effects in the rabbit.

In the mouse daily doses of hydracrylate equivalent to 75 per cent of the LD$_{50}$ administered for 38 days produced a 40 per cent rise in platelet count. One half of the LD$_{50}$ was without effect. In the rabbit one third of the LD$_{50}$ of hydracrylate produced platelet effects but one fourth of the LD$_{50}$ did not.

In chronic feeding experiments in which hydracrylate was administered

concentrations Plasma BPL treatment levels ranging from 800 to 6000 mg per liter have been evaluated and in addition during the past year plasma treated with BPL in combination with ultraviolet irradiation has been followed clinically (Table 4)

A total of 414 patients have received 1153 transfusions of treated plasma or a volume of 290 liters (Table 4) A high percentage of the recipients were children and 85 per cent of the infusions were treated with BPL concentrations of 3 5 Gm per liter or higher No evidence of acute or chronic toxicity or cases of serum hepatitis has been encountered Administration of plasma treated with the BPL ultraviolet combination has been uneventful

Proximity to toxic levels of degradation products can only be detected by studying the maximum volumes of plasma administered with respect to the dosages of these materials present (Table 5) The maximum total plasma dosage administered in a single hospital admission was 360 ml per kg (treated at 5500 mg per liter of BPL) which was given over a 50-day period to an infant 11 weeks of age (Table 5) This also represented the maximum total dosage of degradation products administered — 1386 mg per kg of hydracrylate and 39 mg per kg of β chloropropionate However the highest daily dosage of the degradation products was received by a two and one half year old nephrotic who was given 271 mg per kg per day of hydracrylate and 7 8 mg per kg per day of β chloropropionate for days in plasma treated with 4500 mg per liter of BPL (Table 5)

Although large total doses of hydracrylate have been administered equivalent to almost 70 per cent of the animal LD_{50}, it is evident that the maximum 24 hour dosage in man has not yet reached 15 per cent of that figure No platelet effects have been observed in the human or dog

It is not really feasible to calculate a Therapeutic Index for BPL treated plasma because the effective virucidal concentration against the hepatitis virus is not known However if one assumes an effective treatment level of 4500 mg per liter 2 liters of plasma should be able to be administered to a 70 kg man within a 12 hour period with an excellent Therapeutic Index of 21 (Table 6)

III The In Vivo Fate of the BPL Degradation Products

With the gradual elevation of the BPL treatment level and the administration of treated plasma in patients with possible pre existing liver or kidney damage it became essential to investigate the degradation products in terms of their distribution in the body and their sites of detoxication and modes of excretion

Urinary excretion of the BPL degradation products When the excretion of sodium β chloropropionate was studied it was discovered that there is

TABLE 3

MULTIPLE BETA-PROPIOLACTONE TREATED PLASMA TRANSFUSIONS IN ANIMALS

Plasma Dosage	*Human Equivalent Dosage*	*BPL Treatment Level*	*β Chloropropionate mg/kg*	*Hydracrylic Acid mg/kg*	*Hydracrylic Acid mg/kg/day*
		MOUSE			
900 cc/kg (15 days)	63 L	1500 mg/L	27	945	63
600 cc/kg (11 days)	46	2000	26	840	76
660 cc/kg (11 days)	46	4000	53	1680	153
540 cc/kg (9 days)	38	6000	65	2268	254
		RABBIT			
450 cc/kg (1 mo.)	32	2000	18	630	21
225 cc/kg (9 days)	16	4000	18	630	70
		DOG			
144 cc/kg (20 days)	10	6000	17	605	30
504 cc/kg (168 days) (Puppies)	35	3500	35	1235	7

TABLE 4

BETA-PROPIOLACTONE TREATED PLASMA TRANSFUSIONS IN MAN

March 17 1951–July 9 1956

BPL Treatment Level	*Total Plasma Administered*	*No. of Patients*	*No. of Transfusions*	*Average Plasma Volume Per Transfusion*	*Average Plasma Volume Per Patient*
mg/liter	*liters*			*ml*	*ml*
800–1000	98.4	69	148	665	1426
2000–2500	16.1	13	37	435	1239
3500	33.5	58	217	154	577
3500 + U.V.	24.1	31	87	277	777
4500	56.0	117	393	143	4[illegible]9
5500	44.5	87	197	221	512
6000	17.0	39	74	230	436
Totals	289.6	414	1153		

TABLE 5

MAXIMUM DOSAGES OF BETA-PROPIOLACTONE DEGRADATION PRODUCTS ADMINISTERED TO MAN IN TREATED PLASMA

March 17 1951–July 9 1956

BPL Treatment Level mg l	Age of Patient	Maximum Plasma Dosage ml kg	Hydracrylate Dosage			B Chloropropionate Dosage		
			mg kg	mg kg try	No of trys	mg /kg	mg /kg / try	No of trys
2500	—	81 5	143	48	3	4	1 3	3
3500	Premat Infant	1 9	319	78	4	9	2 3	4
3500 + U V	64 yrs	93 5	2 9	229	10	6 5	7	10
	60 yrs	42	103	103	1	2 9	2 9	1
4500	2½ yrs	172	542	271	2	15 5	7 8	2
5500	11 weeks	360	1386	146 max	50	39 0	4 1 max	50
	14 mos	264	1015	175 max	8	29 0	5 1 max	8
6000	2 yrs	72 1	303	30	10	8 7	9	10
	4 mos	24	101	101	1	2 9	2 9	1

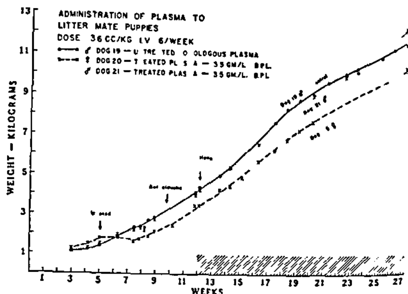


Figure 8

an in vivo conversion of this compound to hydracrylate Within 10 hours approximately 60 per cent of a large intravenous dose of sodium β chloropropionate is excreted but one half of it only after undergoing conversion to hydracrylate (Figure 9)

Excretion plays an important role in the fate of large intravenous doses of sodium hydracrylate (Figure 10) Excretion of one third of the LD$_0$ in the dog is very rapid 4 per cent of the total dose appearing in the urine within hours Excretion is essentially complete at 10 hours and accounts for approximately 77 per cent of the administered dose (Figure 10) At such high dosages metabolic transformation cannot account for more than 10 to o per cent of the administered dose

Comparative rates of hydracrylate excretion were studied in 2 species along with the effect of dosage size on excretion rate The excretion rates of 4 different intravenous doses in the dog ranging from 259 to 700 mg per kg are shown in Figure 11 Plasma decay curves for one dog (number 26) are plotted following administration of both a large and small dose on separate occasions Also included are the excretion and plasma concentration curves of a three fourths LD$_0$ of hydracrylate in the mouse

In the dog it was found that regardless of dosage approximately 50 per cent of the dose of hydracrylate was excreted in two and one half hours (Figure 11) The percentages of the total dose accounted for by excretion range from 58 to 77 per cent with lower total recoveries for the smaller

URINARY EXCRETION OF I.V. ADMINISTERED
SODIUM B-CHLOROPROPIONATE IN THE DOG

SODIUM HYDRACRYLATE

SODIUM B-CHLOROPROPIONATE

EXCRETION — % OF TOTAL DOSE

HOURS

FIGURE 9

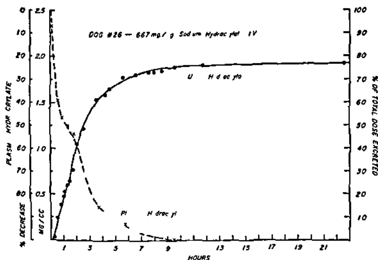


FIGURE 10 Relation between excretion rate and plasma concentration in the dog

Table 6

MARGIN OF SAFETY

Plasma Volume	Blood Treatment Level	Weight	Calculated Therapeutic Index
2 liters/12 hr period	4500 mg /l	70 kg man	71 (Based on dog LD_{50})

dosages The initial excretion rate is much more rapid in the mouse than in the dog and 50 per cent was excreted in the first hour [17]

Renal clearance studies were performed in the dog by the usual constant infusion techniques The excretion pattern of hydracrylic acid was found to closely resemble that of urea Its molecular weight and diffusion constant lie between urea and creatinine At flow rates below 1 5 ml per M per minute the hydracrylate clearance is approximately proportional to the square root of the flow With larger urine flows the rate of excretion is directly proportional to the plasma hydracrylate concentration At a urine flow of 1 0 ml per M per minute the clearance in the dog is 48 ml per M per minute while urea is 41 and creatinine 84 However hydracrylic acid clearances can reach figures as high as 90 becoming equal to the creatinine clearance whereas urea never approaches the glomerular filtration rate

Tissue distribution of hydracrylate Blood and tissue distribution studies were carried out in mice sacrificing animals at various intervals following an intravenous hydracrylate dosage equivalent to three fourths of the LD [50] Time concentration curves were obtained simultaneously for blood kidney brain spleen and liver (Figure 1) In each case the blood hydracrylate is represented by a line and the respective tissue by a dot All tissue drug levels reproduce in general the slope of the blood decay curve However the compound tends to concentrate in the kidney at levels higher than those in blood Results were also expressed as tissue blood concentration ratios in order to determine whether a given tissue tended to differentially remove and concentrate the agent It will be seen (Figure 13) that throughout the entire excretion period of hydracrylate kidney levels invariably exceed the simultaneously determined blood concentrations [17] A constant liver blood hydracrylate ratio is established within 4 minutes after injection the liver concentration being only 0 6 that of blood

The blood and tissue distribution of sodium hydracrylate in the mouse is summarized in terms of percentage of the total dose administered The distribution curves for urine blood and tissues are plotted for the first 3

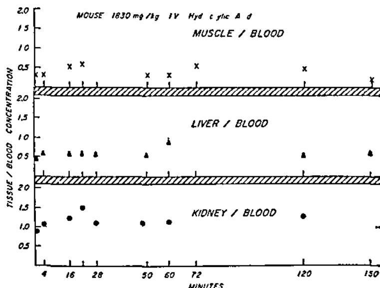


FIGURE 3 Effect of time after admin tration on mouse tissue blood hydra crylate concentr t on rat es

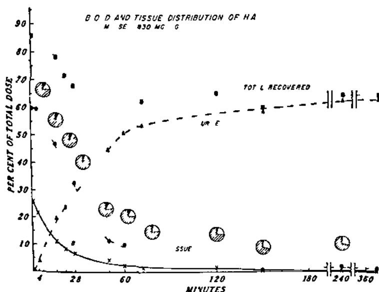


FIGURE 14 Bl d nd t d trilut n of sod um hydr crylate th time (e pr se l in t rn f g t g f the t tal d e a lm n tere l to mice)

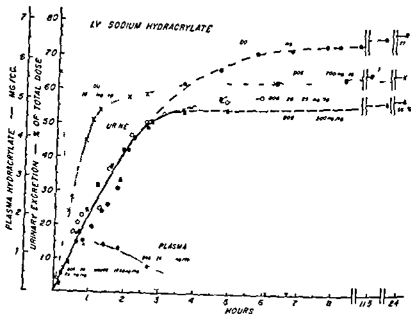


FIGURE 11 Effect of species and dosage size on excretion rates of hydracrylic acid

MOUSE 830 mg/kg HA

MOUSE 1830 mg/kg HA

SODIUM HYDRACRYLATE — Mg./Gm. Tissue

BLOOD KIDNEY

BLOOD MUSCLE

BLOOD LIVER

BLOOD SPLEEN BRAIN

MINUTES

FIGURE 12 Time concentration curves for hydracrylic acid obtained simultaneously for blood and 5 tissues in the mouse

TABLE 7

EFFECT OF SODIUM HYDRACRYLATE ON SURVIVAL TIME FOLLOWING BILATERAL NEPHRECTOMY IN THE MOUSE

Group	*Mean Survival Time ± Standard Error*	*t Value*	
		Mean	*Difference between Means*
Bilateral Nephrectomy + Saline	16.6 Hrs (±1.1[illegible])	14.83	0.16
Bilateral Nephrectomy + Sod. Hydracrylate DOSE 3000 mg/kg (Acute LD) (2 hrs post-op.)	17.15 (±3.18)	25.39	

(1) Beta propiolactone hydrolyzes rapidly and completely in aqueous solutions.

(2) The end products of its hydrolysis are structurally related to lactic acid, *are of low toxicity and show little species variation.*

(3) The major degradation product, hydracrylic acid, is both metabolized by the liver and excreted. With large intravenous doses, rapid excretion can account for as much as 80 per cent of the total dose. Large doses have a diuretic effect, and with high urine flow rates the excretion rate is directly proportional to the plasma drug concentration. These factors account for the striking lack of cumulation displayed by this compound.

(4) It has also been demonstrated that there is an additional margin of safety in the fact that the liver is capable of metabolizing increased amounts of hydracrylate in the presence of renal insufficiency.

During the past five and one half years, a total of 414 patients have received 1153 transfusions of beta propiolactone treated plasma, or a total of 290 liters. No evidence of acute or chronic toxicity, sensitization, or cases of serum hepatitis have been encountered.

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2 Gresham, T. L., Jansen, J. E., and Shaver, F. W.: B-Propiolactone. IV. Reactions with salts of carboxylic acid. *J. Am. Chem. Soc.* 70: 1003, 1948.

postinjection hours (Figure 14) In this figure each point on the tissue curve is accompanied by a small reference circle whose segments indicate the percentage composition of the point in terms of the 3 tissues comprising it The kidney segment is black liver white and muscle hatched Immediately after administration 86 per cent of the total dose can be recovered in the blood kidney liver and skeletal muscle tissues which comprise only 55 per cent of the body weight of the mouse At this point the tissues contain 60 per cent of the total dosage 47 per cent of which is in muscle (Figure 14) Urinary excretion begins immediately and continues until 65 per cent of the total dose has been eliminated Throughout the entire period muscle contains the greatest percentage of the total dose because of its larger mass The liver becomes relatively more important toward the end of the period when excretion is nearing completion The upper dotted line of the graph indicates a final recovery of 65 per cent of the total dose from the three tissues blood and urine Higher initial recoveries are obtained when the whole homogenized mouse is employed but difficulties are encountered from high blanks in the chemical analysis

Metabolism of hydracrylate With one third of the LD_{50} of hydracrylate we have seen (Figures 10 11) that the dog excretes approximately 80 per cent of the total dose At such dosages metabolic transformation cannot account for more than [illegible]0 per cent of the administered hydracrylate However metabolism appears to be much more important than excretion in the fate of small doses Studies of urine hydracrylate concentrations during long term oral feeding in mice revealed that at low blood levels only 2 to 5 per cent of the dose is excreted Approximately 10 per cent of the small dosages administered to man in treated plasma are excreted Even with large intravenous doses we have seen that the dog excretes less of the total dose as the dose is lowered (Figure 11) In addition survival times of bilaterally nephrectomized mice receiving lethal doses of hydracrylate do not differ significantly from operated saline controls (Table 7) This suggested that some other organ was involved in the fate of this agent which was capable of assuming more than its usual share of the burden in the presence of renal insufficiency The liver was more directly implicated by the fact that the LD_{50} dose of hydracrylate is significantly reduced in the presence of experimental liver damage[14] In addition incubation of this degradation product with mouse liver slices results in a 30 per cent disappearance of drug in 8 hours

Summary

Certain characteristics of the aqueous reactions of beta propiolactone and the properties of its end products combine to make this compound ideally suited toxicologically as a sterilizing agent for biologicals

33

*Combined Beta-Propiolactone and Ultraviolet Irradiation for Plasma Sterilization**

FRANK W HARTMAN M D and GERALD A LoCRIPPO M D
(Detroit Michigan)

The use of more than one agent in any method of sterilization is based on sound experimental and practical experience The combination of phenols with heavy metals the application of two or more antibiotics and the use of various physical agents with chemicals constitute but a few well established examples of the added effectiveness of the multiple approach in sterilization

The application of such combined methods is especially appropriate in situations where selective action on the infectious agent and minimal effect on the material to be sterilized such as skin bacterial and viral vaccines tissue graft and blood or plasma is desired

In the instance of blood plasma it is important not only to destroy the infectious agents and avoid denaturization but at the same time to obtain an end product essentially free of toxicity As far as chemical agents are concerned the margin between the virucidal concentration and toxicity will be narrow or absent unless they are degraded rapidly and completely to much less active substances during the process of sterilization

Of the 600 compounds screened [1] in the last five years in the course of our investigations only 6 per cent were effective with one or more viruses and only 1 per cent were effective against a battery of test viruses in blood serum

When the most promising chemicals were evaluated with all the criteria considered using lymphocytic choriomeningitis eastern equine encephalomyelitis and the MM strain of encephalomyocarditis as the battery of test viruses it was found that there was no uniformity in activity Such variability indicates that each virus must have its inactivation de

* Assisted by grants from the Atomic Energy Commission Department of Defense Department of the Army Chemical Corps and the Research and Development Division Department of the Army

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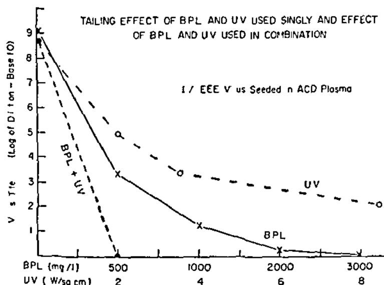


FIGURE 1

is a marked tailing effect observed in the attempt to reduce the virus activity to nondetectable quantities when each agent is used alone A concentration of 500 mg per liter of BPL combined with 2 milliwatts per sq centimeter of ultraviolet irradiation (using the Dill apparatus) reduced the virus activity to the base line zero whereas BPL alone required a concentration of 3500 mg per liter and ultraviolet alone required greater than 8 milliwatts per sq centimeter When BPL and ultraviolet are combined the latter concentrations can be used without significantly altering the plasma proteins This increases markedly the margin of safety since the virucidal concentration of the combination which will reduce the virus activity to the base line zero is 7 times lower than that of BPL alone and 3 to 4 times lower than that of ultraviolet alone

Electrophoretic patterns of human ACD plasma were made before (Figure) and after (Figure 3) treatment with the above concentrations of BPL (3500 mg per liter) and ultraviolet irradiation (3 sterilamps G36T6 in the Dill apparatus) The fibrinogen peak remains well defined and the albumin and globulin patterns are not significantly altered This seems to indicate that the treatment of plasma with combined BPL and ultraviolet has additive and often synergistic virucidal action without increased alteration of the protein constituents

Sterilization of large volumes of plasma artificially infected with MM virus and *Aerobacter aerogenes* bacteria was attempted The purpose of

termined individually making the use of human volunteers essential to obtain conclusive data regarding the hepatitis viruses

It has been determined that beta-propiolactone (BPL) is the least toxic of all the most effective chemicals and further it is rapidly and completely hydrolized Beta propiolactone was first synthesized by Johannson in 1915 using the silver salt of beta iodo propionic acid but Beckurts and Otto attempted to make it in 1885 Kung in 1941 found a method of synthesis of BPL from ketene and formalin and assigned the patent to B F Goodrich Company Since that time the material has been studied and produced by Dr Gresham and his associates in the research center of the Goodrich Company In 1950 BPL was furnished to us by Parke Davis and Company for evaluation in our screening All subsequent BPL was obtained through the courtesy of Dr Gresham and his company

This chemical is a colorless stable liquid in its concentrated form but quite unstable in aqueous solutions It is best kept at – 0° to –30 in plastic or neutroglass sealed containers It has a specific gravity of 1 149 and is soluble in water at 5° C It reacts readily with hydroxyl amino carboxyl sulfhydryl and phenolic groups

In pure form BPL is caustic and toxic but it hydrolyzes in hours at 37° C to sodium beta propionate and sodium hydracrylate both of which are relatively nontoxic[4] Due to this fact the toxicity of BPL proper does not have to be considered in the sterilization of plasma

The process of virus inactivation with BPL is rapid and complete when hydrolysis is finished if the chemical is used in proper concentration a proper temperature The acidity of plasma to which BPL is added rapidly increases therefore the repeated or continuous addition of sodium hydroxide is utilized to maintain the pH between 6 8 and 7 4 for the best preservation of the proteins

Although plasma treated with 0 6 per cent BPL concentration is not toxic to man upon intravenous administration this concentration is 1 5 times that necessary to inactivate the most resistant laboratory virus (eastern equine encephalomyelitis) and 1 times that necessary to inactivate the most sensitive virus (lymphocytic choriomeningitis) when these agents are seeded in plasma However in order to maintain the integrity of the plasma proteins a concentration of 0 45 per cent BPL *should not be exceeded Since this concentration is only slightly in excess* of that necessary to inactivate the most sturdy laboratory viruses it may not be sufficient to inactivate the trace quantities of hepatitis virus that may be theoretically present For these reasons, the combination of BPL and ultraviolet irradiation previously reported [1-6] has been extensively studied and the technique for the treatment of large volumes elaborated

The sharp contrast between BPL and ultraviolet used singly and the synergistic effect of the combination is demonstrated in Figure 1 There

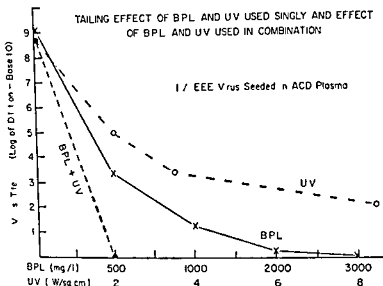


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Although plasma treated with 0.6 per cent BPL concentration is not toxic to man upon intravenous administration this concentration is 15 times that necessary to inactivate the most resistant laboratory virus (eastern equine encephalomyelitis) and 1 times that necessary to inactivate the most sensitive virus (lymphocytic choriomeningitis) when these agents are seeded in plasma *However in order to maintain the* integrity of the plasma proteins a concentration of 0.45 per cent BPL should not be exceeded Since this concentration is only slightly in excess of that necessary to inactivate the most sturdy laboratory viruses it may not be sufficient to inactivate the trace quantities of hepatitis virus that may be theoretically present For these reasons the combination of BPL and ultraviolet irradiation previously reported[-9] has been extensively studied and the technique for the treatment of large volumes elaborated

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this study was to demonstrate that large volumes of heavily infected plasma could be sterilized by the technique presently being used to process our plasma for clinical use Eight liters of human plasma were seeded with 1 per cent suspension of MM virus (mouse infected brain tissue) and 3 million organisms (*Aerobacter aerogenes*) per milliliter of plasma and treated with BPL and ultraviolet irradiation in combination This experiment simulates production volumes of plasma in which the chance of incomplete sterilization is increased due to the problems involved These are (1) inadequate mixing of BPL mixtures and ( ) variation in ultraviolet intensity over long periods of irradiation as well as inadequate penetration of protein particles in thick films

The results of this experiment are seen in Table 1

Table 1

8 LITERS OF PLASMA SEEDED WITH MM VIRUS AND *AEROBACTER AEROGENES* STERILIZED BY TREATMENT WITH BETA-PROPIOLACTONE (BPL) AND ULTRAVIOLET (UV) IRRADIATION COMBINATION

Condition of Plasma Treatment	Results	
	Virus Titer (LD)	*Bacterial Count per Milliliter*
1 Non treated control	6 5	3×10^3
2 BPL (3500 mg per liter) before UV (1 hr at 6–9 C)	0 2	6 × 10[illegible]
3 BPL plus UV irradiation †		
a) Immediately after irradiation at 9 C	0 0	0 0
b) Total volume brought to 37 C	0 0	0 0
4 4 hours after treatment	0 0	0 0
5 7 days after treatment	0 0	0 0
6 28 days after treatment	0 0	0 0

Reciprocal of negative log of virus dilution

† Intensity of irradiation (3 sterilamps G36T6 in a Dill Apparatus) 11 3–11 9 milliwatts per sq cm

BPL at a concentration of 3500 mg per liter at 6 to 9 C for 1 hour reduced the virus titer and the bacterial count to small quantities before ultraviolet irradiation was given It must be emphasized that at this temperature and for this duration of treatment only 10 to o per cent of the potential value of the drug has been used After ultraviolet irradiation 70 to 80 per cent of the drug is still present to produce additional

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effect upon possible trace quantities of the pathogens present However as can be seen in Table 1 (Number 3) the plasma is sterile at this point in the procedure The additional 70 to 80 per cent of undegraded BPL can then serve as an added margin of safety for reacting with undetectable quantities of virus particles *if necessary* This is accomplished when the plasma is brought to 37° C and the BPL residual is fully hydrolyzed Seventy two to 96 hours at 4° C would be required to attain the equivalent degree of reaction and hydrolysis However the more rapid rate of reaction is recommended for the purpose of neutralizing the acid products of hydrolysis as they *form and thus maintain a range in pH levels between* 6 8 and 7 4 This renders the procedure not only safer but more economical and feasible in production processing In Table 1 (Numbers 4 5 and 6) we can see that sterilization is complete and virus reactivation cannot be *demonstrated when assayed at 4 hours and after 7 and 28 days of* storage

The synergistic effect of BPL and ultraviolet combination can be demonstrated by the inactivation of *Escherichia coli* phage (T_3 strain) seeded in plasma The additive or synergistic effect of two virucidal agents is often difficult to demonstrate when small quantities of each agent are capable of inactivating the bulk of the virus particles to almost nondetectable quantities Although the animal viruses respond in this manner to *BPL* concentrations and ultraviolet irradiation when used separately the tailing effect of these agents in producing complete sterilization has been useful for demonstrating their potential value when used in combination However the problem of nondetectable trace quantities of virus particles remains a hazard when the test animal for virus assay is not sensitive enough for such detection Certain bacterial viruses on the other hand can be detected even when only one virus particle persists as in T_3 phage for the organism *E coli* This action is emphasized when the plaque count per unit volume is made before and after the virus seeded plasma is treated in the following ways (1) with varying intensities of ultraviolet irradiation ( ) with increasing concentrations of BPL and (3) with the lowest concentrations of each agent used in combination Data in Table show the synergistic action of *BPL and ultraviolet in* combination on T_3 phage The total effect with the combination is appreciably greater than the additive effect of each agent used separately

Reactivation may occur with certain viruses upon storage but this is not so with the T_3 phage *Table also shows that after 8 days of storage* the total virus count decreased indicating additional inactivation of the virus particles in both the treated and untreated material rather than reactivation This is also true of the two animal viruses studied in this manner namely *the eastern equine encephalomyelitis virus and the* MM virus

TABLE 2

EFFECT OF BETA-PROPIOLACTONE (BPL) AND ULTRAVIOLET IRRADIATION ON *E COLI* (T) PHAGE SEEDED IN 90 PER CENT ACD PLASMA WHEN TREATED SEPARATELY AND IN COMBINATION

Ultraviolet Irradiation		*BPL Concentration*	*Plaque Count per Milliliter*	
Number of sterilamps	*Milliwatts per sq cm*	*mg per liter*	*2 days storage*	*?8 days storage*
None	—	None	10 × 10	? × 10
1	3 1	None	1 × 10	? × 10
2	6 4	None	3 × 10	1 × 10⁵
3	7 8	None	× 10	? × 10⁴
None	—	500	1 × 10	2 × 10
None	—	1000	3 × 10	16
None	—	1500	6 × 10	0 0
None	—	2000	5 0	0 0
None	—	500	0 0	0 0
None	—	3000	0 0	0 0
1	2 6	500	2 × 10³	40
1	2 7	1000	10	0 0

LARGE VOLUME STERILIZATION OF HUMAN ACD PLASMA BY BETA PROPIOLACTONE AND ULTRAVIOLET IN COMBINATION

Procedure In the processing of production volumes (8 to o liters) of plasma using the BPL and ultraviolet treatment the procedure has not been altered from the standard (National Institutes of Health) specifications established for the ultraviolet method The major problem in the combined treatment consisted of experiments to ascertain (1) when in the ultraviolet method to best add the BPL ( ) how to adequately mix the drug volume into the large plasma volume before BPL would hydrolyze to any appreciable amount and (3) how to best control the rate of BPL hydrolysis so that the acid products could be neutralized within a narrow range of pH variation (6 8 to 7 4)

It was found that it is best to add BPL after pooling and clarification of the plasma and before ultraviolet irradiation In order to add the drug with minimal hydrolysis the plasma is kept at refrigerator temperature (4 to 6 C) It is then placed on a mechanical shaker to produce gentle splashing Before BPL can be added it is necessary to raise the pH of the ACD plasma from 6 8 to 7 8 This is done by the addition of sterile normal sodium hydroxide through a closed system This rise in pH is sufficient to take care of the acid products of BPL hydrolysis while the drug is being added and the mixture treated by ultraviolet irradiation

BPL is then added in the cold during splash shaking Since the half life

of BPL hydrolysis is 16 to o hours at 4° C., this permits ample time for thorough drug plasma mixing and passage of the plasma through the ultraviolet apparatus before BPL hydrolyzes to any appreciable amount (not more than 10 to o per cent) During ultraviolet treatment BPL plasma mixture is kept cold in ice containers As shown in Table 1 both virus titer and bacterial counts are reduced to relatively small quantities before ultraviolet treatment

After ultraviolet irradiation the plasma is collected directly in a sterile bottle which is then placed on the mechanical shaker This collection bottle has the following attachments (1) a thermometer ( ) a special needle for slow addition of normal sodium hydroxide and (3) a special tube and pumping arrangement for constant plasma flow through the pH meter (a closed and sterile system)

After all of the plasma has been collected and splash mixing begun, the mixture has a temperature of 1 to 14 degrees C. and a pH value of 6 6 to 6 9. To increase the rate of BPL hydrolysis and enhance its virucidal activity two infrared lamps are used to raise the temperature to 37 C. As the temperature is raised the rate of hydrolysis increases and the pH of the mixture is lowered proportionately Sodium hydroxide must be added at a rate parallel to the BPL hydrolysis in order to maintain the indicated range of pH variation

When the calculated amount of sodium hydroxide equivalent to the total amount of BPL has been added and the temperature has reached 37 C. (approximately to 3 hours for 8 liters of plasma) the plasma is bottled in units of 5o ml volumes These are stored at 4 to 6 C. Sterility and pyrogen tests are completed before the plasma is used clinically

Table gives a summary of the effect of BPL and ultraviolet treatment on the plasma proteins The source of plasma is from our hospital blood bank and consists of leftover material following the administration of packed erythrocytes

In the six lots of plasma treated for clinical use the volume varied from 5 5 to 8 liters and the duration of storage before treatment ranged from 1 to 8 months With the exception of lot 1 all lots were treated with 3500 mg per liter of BPL and 3 ultraviolet sterilamps (C36T6 in the Dill UV apparatus) as described above The effect on the plasma proteins is reflected in the percentage of fibrinogen albumin and globulin fractions found in the electrophoretic pattern There appears to be some correlation in the duration of plasma storage and the lability or disappearance of the fibrinogen pattern In lots 4 5 and 6 the fibrinogen peak was maintained after treatment in 3 of the 4 lots processed In the plasma which had been stored before treatment for 6 to 8 months (lots 1 and 3) the fibrinogen appeared low before treatment and absent after treatment This is particularly evident in lot 3

TABLE 3

EFFECT OF BETA-PROPIOLACTONE (BPL) AND ULTRAVIOLET (UV) TREATMENT IN COMBINATION ON PLASMA PROTEINS

Lot No	Plasma Volume (liters)	Duration of Storage (months)	BPL + UV	Total Proteins (gm %)	Paper Electrophoresis Patterns Percentage of Total Proteins: Fibrinogen	Albumin	Globulin
1	5.5	6	None	5.3	14.3	54.4	31.3
			4500 + 11	4.7	0.0	52.3	4[illegible]
2	7.0	2	None	5.[illegible]	15.4	50.4	34.[illegible]
			3500 + 10.5	5.0	20.0	46.6	33.4
3	8.0	8	None	5.3	6.4	44.4	49.0
			3500 + 11	4.8	0.0	43.0	57.0
4	8.0	1	None	5.3	19.0	44.4	36.6
			3500 + 13	5.0	8.9	40.6	50.5
5	7.6	2	None	5.9	17.4	39.9	4[illegible]
			3500 + 10	5.2	0.0	41.4	58.6
6	7.3	1	None	5.6	16[illegible]	48.9	34.9
			3500 + 10.[illegible]	5.1	0.[illegible]	48.6	31.[illegible]

BPL is expressed in mg per liter and UV in milliwatts per sq cm

The final product is clear and amber, stores well at refrigerator temperature and can also be lyophilized and readily reconstituted. The fact that 3500 mg per liter of BPL in combination with standard ultraviolet irradiation is in marked excess of that necessary to inactivate the sturdiest of the laboratory viruses (eastern equine encephalomyelitis) and still maintain the integrity of the fibrinogen electrophoretic pattern suggests that this combination would be the most promising for the inactivation of the hepatitis virus in human plasma.

The combination method for plasma sterilization was instituted in our blood bank in January 1956. This method replaced the procedure using 6000 mg per liter BPL alone. Over a 6 month period 31 patients received 87 transfusions treated with 3500 mg per liter BPL plus ultraviolet irradiation. The recipients in this series averaged 16 years in age ranging from 1 day to 66 years. The total volume per patient averaged 8[illegible]0 ml. The maximum total volume was 8,[illegible]00 ml administered in 10 days. In terms of ml per kg per [illegible]4 hour dose the minimum was [illegible] ml per kg and the maximum was 4[illegible] ml per kg. The latter patient received [illegible]500 ml of plasma in an effort to control shock associated with a gangrenous leg. At autopsy [illegible]4 hours later no toxicity attributable to BPL was found.

R. J., a 64 year old male with uremia, pyohydronephrosis and hypoproteinemia received 93.5 ml per kg of plasma in a 10 day period. There were no acute toxic manifestations and at autopsy there were no histologic findings to suggest any toxicity of the rather large amounts of treated plasma received in the 1[illegible] days prior to death. The remaining

patients in this series have been observed for 5 months or more with no evidence of chronic toxicity or hepatitis

Obviously the number of cases and the duration of study prevent any conclusions at this time Until the combined method can be tested on known infected hepatitis plasma and evaluated in human volunteers we shall continue to appraise the procedure clinically in the manner described above

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DESIGNATED DISCUSSION

GILBERT DALLDORF, M.D. (Albany, New York): I would think there would be many here who would be somewhat surprised that we gave so much attention to sterilizing methods. We began the day's session by talking about methods that we know are effective. Certainly there is great value and also safety in the blood fractions that have come out of the elegant and complete work at the Department of Physical Chemistry at Harvard. Other methods are also effective in controlling serum hepatitis in medical practice.

I thought of that in hearing Dr. Gibson and what he had been able to accomplish. I know of small blood banks where hepatitis usually is controlled because they are so careful about their donors. Dr. Strumia's blood bank in Bryn Mawr is one like that. He has the pedigree of all his donors and he does not have cases of hepatitis. We have also heard of room storage which is effective.

The answer, of course, is that what is wanted, and wanted especially by the armed services and Civil Defense and other people, is a magic bullet, and that is something which can be used under the special requirements of the military, or in time of disaster, or that can be used more carelessly. That is what the effort has been toward finding, namely, a magic bullet.

Fortunately we are blessed because we have both the chemists and the physicists interested now. I see they are to join forces and give us a combined treatment which also looks very promising.

The doubts I have on that point arose only when I saw Dr. Pollard's interpretation of a phage, which to me looked like the kind of infernal machine you see in an old laboratory at General Electric, except it had no wires on it. The organic chemist in my laboratory, when she draws phage, always makes it look like a carbon ring with a tail on the end, and when I draw it, it always looks like a spermatozoon. Dr. Pollard should know that the first man to see phage said it looked like a spermatocyte. At any rate, that is the problem.

We are looking for something more effective than the methods we have, and that can be worked but not under all those situations. It is a practical problem in this sense, and it is also practical now, too, because the real obstacle is the question of actually determining how effective these methods are in the case of serum hepatitis — in other words, the testing. Everyone is waiting for a reasonable solution to that problem. I don't see how we can proceed without it. Dr. Pollard raised the point himself in the discussion.

GENERAL DISCUSSION

ALLEN TAYLOR PH D (Detroit Michigan) I would like to add a word to Dr LoGrippo s and Dr Hartman s presentations on the combined use of a chemical and ultraviolet inactivation method Over the past two years at Parke Davis we have been putting such a combination to practical use The accumulative inactivation of formalin ultraviolet on poliomyelitis virus was tested The control preparation #1 was diluted and titrated in bottles and the method of assay is plaque formation Plaques were read on the third day and again on the seventh day

Simple dilution does not produce any delay in plaque formation All of the plaques potentially available in this material are visible on the third day You get essentially a ratio of 1 However where two increments of ultraviolet were used and this is two 7 watt increments of ultraviolet used in succession the titer dropped about 2 logs per increment of ultraviolet Here again you see that there is very little change in the plaque ratio following the ultraviolet treatment

If two 1 hour increments of formaldehyde inactivation are used the titers drop to about the same degree but there is a marked delay in the cytopathogenic effect of this virus Only 15 plaques are shown on the third day and only 168 on the seventh day The ratio is 11 compared to 1 for control material

If you use combinations of ultraviolet and formalin you get a slightly higher value than you do for the control or the ultraviolet alone Formalin used first followed by ultraviolet gives again an intermediate ratio somewhat higher than the other The important thing here is that one type of inactivation — ultraviolet — is a physical method You are putting the ultraviolet through a virus particle and the type of inactivation being produced I think has been very well discussed by Dr Pollard There is molecular destruction within the virus particle whereas the formalin or the chemical treatment has to work by diffusion as you will see a little later

These animal viruses are essentially cell like in nature and this is the only way they can act Dr Eugene Timm and his group at Parke Davis have been carrying along combination experiments with beta propiolactone and ultraviolet in conjunction with this The results of the beta propiolactone inactivation alone are similar to the formalin alone results However there is a very definite suggestion that there was a synergistic effect when the beta propiolactone and the ultraviolet were used together The ultraviolet inactivation is working through molecular disruption The ultraviolet absorption curve of untreated poliomyelitis virus is that of a typical nucleoprotein The same virus after treatment with sufficient ultraviolet irradiation is completely inactivated but yet retains antigenicity The major peak at around 260 mμ shifts toward the shorte

wave lengths and there is a marked increase in absorption in the region of the main absorption

Earlier Dr Pollard suggested the use of irradiation and thermal inactivation in combination he suggested that the amount of radiation could be reduced when combined with heat He also presented a picture of an animal virus — an ideal animal virus — as drawn from his experiments

Perhaps I should not have the temerity to say so but I think that an electron micrograph of poliomyelitis virus is an indication of the actual virus which we see in essentially the same form that Dr Pollard has deduced from his irradiation experiments The dense central core we know is composed of nucleoprotein and has electron stopping power In this very lightly shadowed preparation you can see the peripheral areas of the virus collapsed around this central core We feel that not only this virus but animal viruses in general can be considered to be submicroscopic cells and very definitely (as the evidence accumulates) combination methods of inactivation will prove more effective than any one method alone

WALTER N MACK PH D (East Lansing Michigan) I would like to ask Dr LoGrippo if he has tried using beta propiolactone to sterilize serum prior to its use in tissue culture

GERALD A LOGRIPPO M D (Detroit Michigan) We have been using beta propiolactone to sterilize all our serum in the tissue culture laboratory We now have the HeLa cells on the twentieth subline with beta propiolactone treated serum using virucidal concentrations of 0 3 per cent beta propiolactone However when you use 10 per cent of the treated serum in the growth medium the degradation products are lowered to 0 03 per cent You cannot exceed 0 04 per cent of the degradation products without toxic effects on the HeLa cells

When we want to use serum above 10 per cent in the growth medium then we use 0 1 per cent beta propiolactone plus ultraviolet irradiation for serum treatment We can go up to 30 per cent serum in the growth medium and still keep below 0 04 per cent of the degradation products The Detroit 6 line of cells is now in the eighth passage and so are Chang liver cells

We find that beta propiolactone treated serums work very well As a matter of fact they work so well that we are now able to use the pooled serums coming out of our serology laboratory which amounts to as much as 1000 to 1800 ml per week I accumulate about 5 to 6 liters of this serum and then treat it by the combined physical chemical (beta propiolactone and ultraviolet) method Such serum pools represent material from at least 5000 patients and it is really toxic for tissue culture However as long as we filter it and treat it with beta propiolactone we can use it in our tissue culture procedures

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PART V

Differential Diagnosis—Laboratory Methods

Moderator HENRY L. BOCKUS M.D. (*Philadelphia Pennsylvania*)

34

Tentative Classification of Some Current Types of Liver Damage on the Basis of Electrophoretic Serum Analysis

C VIOLLIER M D

(Basle Switzerland)

Electrophoretic patterns of serum protein in liver diseases are not specific but they reveal characteristic changes in protein structure which can be used for differential diagnosis

As references I would like to mention the classical reviews in English by Gutman and Luetscher in German by Schuler and Emmrich in French by Fauvert and in Italian by Murelli

Our studies in this field started with the hepatitis epidemic in 1946 The results of these investigations were published in 1947 by Staub in 1949 and in 1950 by Staub and myself

These are given *in toto* in Table 1 and Table The first table represents the results obtained in 164 patients using the small Tiselius cell and Michaelis s buffer at pH 7 6

As can be seen from Table 1 the benign cases of hepatitis are divided into cases with negative and positive Takata reactions The Takata reaction introduced by Staub in 19 8 was the first flocculation test used in liver diagnosis and is today still of great value in recognizing severe hepatitis Though this reaction is not frequently mentioned in Anglo Saxon literature it has been used in the medical clinic of the University of Basle Switzerland for the past 8 years and is referred to in the following discussion Years of experience have shown that when the Takata reaction is positive the hepatitis is usually of a more severe type Therefore in a large number of cases benign hepatitis demonstrates a constant negative Takata reaction Prevalently positive Takata reactions were found only in 9 to 35 per cent or approximately in one third of the benign cases

However both groups show the same changes in the electrophoretic patterns namely a reduction in the albumin concentration with an increase in beta and gamma globulins The two groups differ only in the degree of protein dysfunction that of the Takata positive group being

...nd of ...ver D sease	Number cases	...bu... %	Globul %					...of Prot %	Al	A/ Quot
			α	α	β	H	γ			
Normal Serum		59		.3			3.		.43	46
...on Adm...on ...on Discharge	35	47 53	3	0 8		.8 0		7 2 7.70		1
...on Admission ...on ...lscharge	30 3	.5 48	5	10 7 10	20 2	3.1	19.3	.36 44	3.0 3 49	.77 93
Ac... ...subacu... Form		44	4.		12	6	23.	40	2 90	79
Infe... ...ous Mononucleos	7	48	4	.2			6.7		3 44	92
Obstruct... ...e Jaundice Chol... ...and Chol... ...thi Tumors ...the ...-Duct	20	42 34		14 0	23	.4 3.5		22 7 00	3.	0 54
...(W... ...)		33	.2		3		3.	5	1	50
a) ... ...Stages of Cirrh. b) ...) ... -Cirrhosis	3 22	36 34.	3		20		23 26	77 38 40	79 2 5	70 52
... C...rh Apparent Recovery		34 37	5		3	5	33 26.4	87 8	34 3.04	2
...the liver a) P... Tumors) Secondary ...umors (Metastases)	5	37	10 10	.5 1		3 7	19	69 80	2.5 5	
... ...umors (...hout Metast...)								36	63	0 70
...liver ...ardiac Insuffi...iency	25	45			5.5	7		20	30	85

TABLE

Survey on the Serum Protein D tribution in a Group of 217 Patients ith Diseases of the Liver or Bile Ducts (Longsworth s buffer pH 8.6)

whereas Weils icterus shows all the signs of an acute infection i e albumin decrease with simultaneous rise of alpha globulin Furthermore it should be stated that the effect of cardiac insufficiency on the liver is negligible according to the electrophoretic diagram

In this summary on liver parenchymal damage I had to generalize for the sil e of brevity It must be remembered however that certain cases will deviate from the rule

With regard to the albumin and fibrinogen decrease observed con stantly in liver diseases the perfusion experiments of Miller and co workers are sufficiently known in this country so as not to require men tion Albumin and fibrinogen seem to be synthesized exclusively by the liver It is thus not surprising that severe functional disturbances of the liver result in a decrease in serum albumin and fibrinogen

The cause of the increase of gamma globulin in chronic liver damage cannot be explained so easily The reasons have been sought in the effect of immunization allergy and hormonal influence (ACTH or adrenal cortex) as well as in the Kupffer cells In our studies the increase in gamma globulin was observed simultaneously with the infiltration of round cells in the portal field

The increase in alpha and beta globulin in cases of obstructive jaundice is still unexplained Greenspan and Mandel have tried to use glucoprotein determinations for differential diagnosis between obstructive and paren chymal jaundice We still do not have enough experience with this

K nd of L ve D sease	Numbe of Cases	bu in %	Globul ns %				T l Pr %	A b %	A/G Quot
			α	β	H	γ			
Normal Serum	1	62 0	9 4	11 9	0	1 7	7 7	4 80	1 63
nos pos 1 Tak neg									
on Adm on	56	52 4	8 1	17 0	0	22 5	7 00	3 66	1 10
on D scharge	2	56.2	8 1	15 5	0	20 1	7 30	11	1 29
2 Takata pos									
on Adm on	23	48 3	6 7	1 9	0 4	27 7	6 93	3 35	0 93
on Discharge	1	40 0	8 1	15	0	26 4	7 50	3 75	1 00
ophy of L ver Ac or ubacute Form	28	4 4	7 1	8 5	3 9	33 1	4.17	2 90	0 90
nfec ous Mononuc os	1	53 1	9 4	10 8	0	2 7	7 85	3 64	1 13
Obstruct ve Jaund									
Chol and Cho l th as	12	49 2	12 1	1 3	0	2 4	4 68	3 28	0 97
Tumors of the B l Duc	7	43 9	1 5	18 3	0	3	86	3 01	0 78
L ptosp os (W D sease)	3	48 0	13 7	13 9	1.3	23 1	7 48	3 59	0 2
C rrhos of L ver									
) F L ve earl Stages of Cirrh.	2	5 0	11	17 3	0	20 3	79	3	1 04
b) Cholang ol C rrhos	3	3		2	0	73 2	13 1	2 37	0 2
) Lae ne Al ohol Cirrhos	10	38	7	17 6	3 0	33 4	7 0	2 72	0 2
on po 1 T one on C rrhosi	7	39 9	9	16 0	3 3	35	5 89	2 39	0 66
2 Apparent Recovery	12		6 2	1 5	0	25 5	80	3 23	0 90
Tumors of the Liver									
) P or Tumors	4	39	10 4	22 8	0	27 7	51	2 55	0 64
b) S ondar Tumors (Me astase)		48	12 7	20 7	0	8 2		2 84	0 94
E ohepa Tumors (or Metas)	4	2 7	17.5	1 6	0	22 2	5 18	2 2	0 75
ve or iac Insuff iency	6	51 2	9.4	15 8	0	23 6	7 00	3 59	1.05
ve Cys	1	47 8	8 3	5 4	0	13 5	7 89	5	1 49
L bs ess	1	3 6	21 2	11 2	0	24 0	90	3 0	0 77

TABLE 1

Survey on the Serum Protein Distribution in a Group of 184 Patients with Diseases of the Liver or Bile Ducts (Michaelis's buffer pH 7 8)

more pronounced In recovery phase both groups show a tendency to return to normal

Table 2 represents the results of electrophoresis in 17 patients using the large cell and Longsworth's buffer at pH 8 6 Here it was possible to separate an alpha 1 and alpha globulin

In this table benign cases of hepatitis are subdivided in the same manner cases with negative or positive Takata reaction The same disturbance in the blood protein pattern can be seen in the two groups i e albumin decrease with rise in beta and gamma globulins

Furthermore you can see from Table that the lowest albumin values are found in cirrhosis chronic hepatitis and liver tumors as well as tumors of the biliary tract In the latter however the gamma globulin is normal or slightly increased whereas the alpha and beta globulin show a strong rise This finding has been used for differential diagnosis

A high concentration of the H component between beta and gamma globulin was first noticed in acute or subacute liver atrophy as well as in advanced cirrhosis and chronic hepatitis Later it was occasionally noticed in other persons who were not suffering from a liver condition In these cases however the H component was never more than 3 to 4 per cent of the total proteins

Infectious mononucleosis behaves like a mild case of benign hepatitis

diagnosis of liver diseases is mainly based on results obtained by the use of the Tiselius apparatus

Practical Aspects

For practical purposes the problems that can be solved by electrophoretic analysis are subdivided into three groups

(1) Differential diagnosis between benign and malignant hepatitis i e between icterus catarrhalis and icterus gravis

(2) Differential diagnosis between obstructive jaundice and hepatitis i e surgical and medical icterus

(3) Differential diagnosis between cirrhosis and liver tumors (metastasis) i e differential diagnosis of the enlarged liver

The answer to problem (1) which every physician must consider when facing liver parenchymal damage is found in Figure 4 and Figure 5 These graphs represent 101 cases of hepatitis from the 1946 epidemic which have been divided into the following two groups

Benign cases presenting the picture of an icterus catarrhalis with congested liver moderate increase in sedimentation rate and fibrinogen and normal serum protein concentration

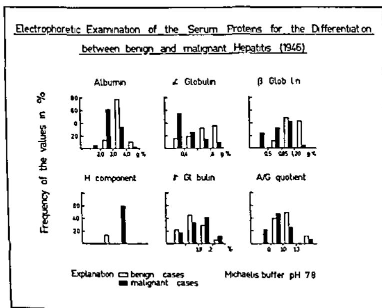


Figure 4

same changes are seen in the proteinogram and lipidogram The thymol turbidity was negative whereas serum iron was markedly increased but returned to normal upon recovery

The third case Figure 3 was a 78 year old male with severe hepatitis The thymol turbidity rose to 25 units and sank upon recovery parallel to serum iron Here too the lipidogram is markedly increased in the first stages of the disease and returned to normal

These examples show the possibilities of paper electrophoresis with the use of special staining methods as an aid in differential diagnosis However in obtaining quantitative information on the globulin distribution the moving boundary method is to be preferred Therefore the following discussion on the practical aspect of electrophoresis in the differential

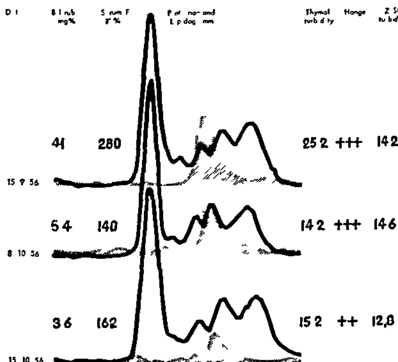


Figure 3

Bili rub. mg %	Sedim. rate m	Fibrinogen mg %	Cholinesterase mm CO_2	T butyrase mm CO_2	Hanger	Thymol turbidity units	ZnSO turbidity units	Takata 4°C	ELECTROPHORESIS Diagram of desc. bound	Albumin %	Globulins % α	α	β	H	γ	Proteins g%	Albumin g%	Quot. A/G
8,65	9/21	186	32,5	18,5	+++	16 0	18,2		24.5.	42,0	3 9	6,8	18 6	4 5	24 2	6,38	2,67	0 72
2 96	—	306	41 9	18 9	+++	19	19 2		4 51	41 6	4 8	6 9	20 0	3 3	23,4	8 25	3 43	0 71
0,85	12/38	302	49 7	25,8	+++	9 5	12,6		23. 51	48,0	5,4	4,7	17 2	2,8	19 7	6 65	3 19	0 92
—	—	—	—	—	—	8,5	11,8		2.6.51	49 3	3 7	8 1	14 4	trace	22,5	8 10	3 99	0 77
—	—	200	45,5	23,5	—	6 8	11,6		24.11.51	52,0	6,1	8 4	14 2	0	17 3	7 44	3 86	1 06
norm.	—	—	—	—	—	6 2	14,5		53.	52 8	5,6	8 4	13 5	0	19 7	7 74	4 09	1 12
H.L. ♀ 33 years																		

Figure 6

the total protein concentration a decreased A/G quotient and a strong positive Takata reaction as well as a delayed sedimentation rate low fibrinogen values and decreased prothrombin time

We will now verify these data by discussing a few typical cases of benign and malignant hepatitis

The first case Figure 6 deals with a 33 year old woman who entered the clinic with icterus of 4 week s duration showing definite signs of parenchymal damage i e positive flocculation tests (Hanger thymol $ZnSO_4$ turbidities and Takata reaction) and an albumin concentration of 42 per cent with a rise in gamma globulin and in the H component A moderate hypoproteinemia was manifested together with a low fibrinogen concentration With daily infu ions of vitamins and levulose the liver tests eventually returned to normal Thirty one weeks after onset of icterus, the albumin concentration rose to 52 per cent the Takata reaction was indefinite but the esterases returned to normal One year after the beginning of jaundice the flocculation test and especially the Takata reaction and the electrophoretic pattern were nearly normal

Figure 7 represents a case of liver atrophy which is characterized by low alpha and beta globulin peaks and the appearance of the H com

Malignant cases presenting the picture of icterus gravis characterized by liver atrophy with ascites edema delayed sedimentation rate decreased fibrinogen and total protein concentrations usually resulting in death

The height of the bars in these graphs shows the observed frequency of the values in per cent The albumin concentration in the benign cases lies around 3 Gm per cent whereas in the malignant cases we usually find a concentration between and 3 Gm per cent And 53 per cent of malignant hepatitis demonstrated an alpha globulin concentration lower than 0 4 Gm per cent whereas in the majority of the benign cases the alpha globulin concentration was above 0 6 Gm per cent As for the beta globulin in the malignant cases its concentration usually was under 0 85 Gm per cent The benign cases demonstrated a concentration of 0 85 to 1 Gm per cent

The H component was found in 6, per cent of the malignant cases whereas in the benign cases it appeared in only 17 per cent

The concentration of gamma globulin in the malignant cases is usually higher than in benign hepatitis This is especially true in patients who have a long standing malignant icterus The A/G quotient is very low in malignant cases frequently under 0 7

The next graph Figure 5 shows the chemical differentiation between malignant and benign hepatitis The malignant cases show a decrease in

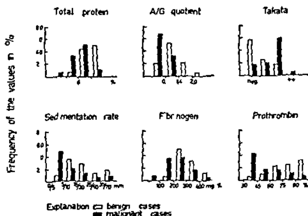


FIGURE 5

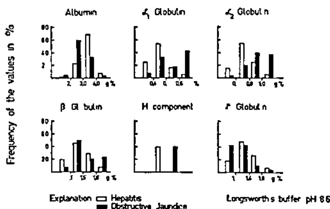


FIGURE 8

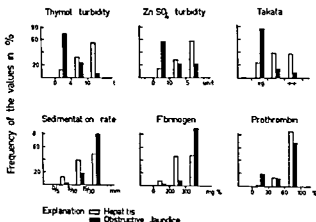


FIGURE 9

Bili rub mg%	Sedim. rate mm	Fibrin ogen mg%	Thymol turbidity units	Ta kata 4°C	ELECTROPHORESIS: Diagramm of desc. bound	Albu-min %	Globulins %: α1	α2	β	H	γ	Pro teins g%	Albu min g%	Quot. A/G
87	4/12	194	120	− + / − + / − + / + +	4 12.50	468	63	87	179	trace	203	700	328	088
98	3/10	138	170	− + / + + / + + / + +	12 12.50	422	74	57	115	79	25,3	528	223	073
117	20/45	162	—	− + / + + / + + / + −	18.12.50	477	4,8	76	5,2	78	24,9	725	346	091

St M ♀ 34-years † 19.12.50 S. Nr 1390

FIGURE 7

ponent The patient a 34 year old woman who had borne four children died within 3 weeks after the appearance of the icterus This occurred 3 months after she had successfully nursed her husband suffering from benign hepatitis We find here hypoproteinemia, a delayed sedimentation rate a low fibrinogen concentration and hypoalbuminemia

In answer to the second problem ( ) differential diagnosis between obstructive jaundice and hepatitis will be attempted on the basis of the third series of graphs Obstructive jaundice is represented in black and hepatitis in white As you can see in Figure 8 the albumin concentration in obstructive jaundice is more often under 3 Gm per cent than it is in hepatitis The reason for this finding is that the obstructive jaundice also includes cases with obstruction due to tumors The decline in albumin in these cases is due to tumor cachexia and not to liver damage

However the increase in both alpha$_1$ and alpha globulin has differential diagnostic value insofar as in benign hepatitis the alpha globulin remains stable The differences are not so pronounced in the case of beta globulin but a very high beta globulin peak is a symptom for obstruction in the biliary tract The H component cannot be used in differentiation between obstructive jaundice and hepatitis as its incidence is approximately the same in both groups

The gamma globulin is of value in that a low concentration is characteristic for obstructive jaundice and a high concentration is more frequently observed in hepatitis

Figure 11 shows a 37 year old woman from the South of France with chronic hepatitis Six weeks before entering the hospital she developed jaundice On examination the liver was not enlarged but all flocculation tests were definitely positive the sedimentation rate was increased and the electrophoretic pattern was similar to that of cirrhosis However laparoscopic examination showed no signs of liver cirrhosis After 6 weeks of hospital treatment consisting of vitamins and levulose infusions the patient showed improvement Four months after the beginning of jaundice the first ambulant checkup showed a slight rise in the albumin but the flocculation tests remained unchanged The second ambulant recheck 7 months after the onset of jaundice gave a definite improvement in the flocculation tests further increase in the albumin and a decrease in the gamma globulin The return to normal of the alpha peak is clearly demonstrated in these diagrams

Finally I would like to mention a rare case of agammaglobulinemia (*Figure 12*) a 16 year old boy with pericarditis endocarditis and purulent pleurisy after secondary infection following varicella Under anti

Bili rub mg%	Sed rate mm	Fibrin ogen mg%	Ha ger	Thymol turbidity units	ZnSO4 turbidity units	Takata 4°C	ELECTROPHORESIS Diagramm of desc bound	Albumin %	Globulins % α1	α2	β	H	γ	Proteins g%	Albumin g%	Quot A/G
3 15	110/127	206	+++	25,8	49 4		15 3. 52	26,1	7 6	9 1	10 9	5 8	40 5	8 70	2 27	0 36
2 71	—	—	+++	24 4	43 4		9 3 5	26 4	8 3	7 4	10 8	10 5	36 6	8 67	2 29	0 36
1 04	103/157	—	—	22,4	49 0		1 3 52	28 6	9 1	9 5	11 6	12 8	28,4	7 83	2 24	0 40
orm	103/127	238	+++	25 1	49 2		21	33 2	7 0	8 5	14 7	8 5	28,1	9 18	3 05	0 50
norm	64/107	—	—	21 2	43,2		16 6 52	39 0	7 3	8 4	16 1	3 6	25,6	9 00	3 51	0 64
0,82	—	—	—	11 0	22 0		1 2	49 1	6 0	7 3	10 5	4 0	23,1	7 20	3 54	0 96
V S ♀ 37 years																

FIGURE 11

Chemical liver tests (Figure 9) further support our electrophoretic observations The thymol and $ZnSO_4$ turbidity and the Takata reaction are more often negative in obstructive jaundice and positive in hepatitis Sedimentation rate and fibrinogen concentration are both increased in obstructive jaundice whereas only a very low prothrombin concentration which rises on injection of vitamin K can be used to recognize obstructive jaundice

In problem (3) differential diagnosis of the enlarged liver can, in the majority of the cases be answered on the basis of the alpha and gamma globulin behavior As can be seen from Figure 10 liver metastasis or primary liver tumors are characterized by an increase in the alpha globulin and the gamma globulin fraction usually remains normal Only after a long period of illness does the gamma globulin fraction rise and in these cases occasionally it gives a pattern which is similar to liver cirrhosis In liver cirrhosis however the alpha globulin is usually normal and the gamma globulin concentration is increased from the start. The biochemical tests too i e thymol $ZnSO_4$ and Takata are more frequently negative in liver tumors than in cirrhosis

A few examples will be given to demonstrate the changes in protein distribution due to chronic liver damage

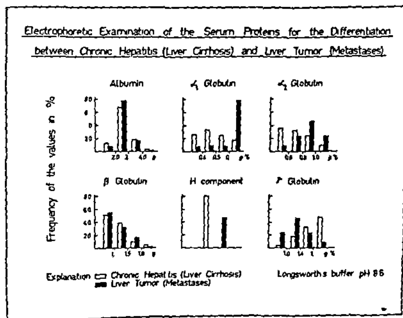


FIGURE 10

nd of ve ase	bum	G obul ns	rote	h nege	Sed ment ion rate
ere acute ve amage V hepa Infec ous mononucl osi	ode decreased	oderat ncreased β and γ g obu ins	normal	ncreased	Increased
reve ac rve amage Ac sube ute ophy of ve	de reased	de ed α and β globul ns, increased and γ g obul	decreased	decreased	delayed
C on ve Damage Fatty ver earl ages of rrh. Porto rrhos Chron hepat i	moderate marked de ased	moderate marked Increased and γ ob	Increased or norma	Increased	ncreased
Obstruct ve Jaund ce hol th and chol cyst Tumors of the b e duc	near normal dec ased	arked ncrease α and β g ob norma γ g obul	orm	Inc ased	I reased
umors the ver Imary tum Secondary tumors of the ve (metastases)	de reased	marked ased α g obu	norma or decreased	Inc ased	Increased
L ve ys	norm	orma	orm l	normal	normal
Liver abscess	decreased	Increased α g obu in	normal	Increased	Increased
L ver cardiac Insuff ency	ght de reased	mode atel eased β g obu	normal	norma	normal

TABLE 3

Directions for the Use of the Electrophoretic Blood Protein Pattern for the Diagnosi of Li er D seases

and beta globulin the appearance of the H component and a marked increase in the gamma globulin fraction A low total protein and fibrinogen concentration plus a delay ed sedimentation rate are characteristic of this type of liver damage

Chronic li er damage which includes fatty degeneration in the early stages of cirrhosis portal cirrhosis and chronic hepatitis discloses a further decline in the albumin with a marked increase in the H com ponent and gamma globulin In comparison to the liver atrophy however the total protein value is normal and the fibrinogen and the sedimentation rate are increased

The obstructi e jaundice shows a decrease in the albumin content especially in cases where the obstruction is caused by a tumor There is also a marked increase in the alpha and beta globulin with normal gamma globulin concentration Fibrinogen and sedimentation rate are raised Of interest are the negative Takata reaction and the low values in the $ZnSO_4$ turbidity or clearing effect of the $ZnSO_4$ reaction according to Heepe

Liver tumors as well as primary and secondary metastases result in a definite decrease in the albumin concentration with a pronounced rise in the alpha globulin

In the case of *li er cysts* the electrophoretic blood protein pattern ob tained is normal

Bili rub. mg%	Sedim. rate mm	Fibrinogen mg%	Takata 4°C	ELECTROPHORESIS Diagramm of desc. bound	Albumin %	Globulins % α	α₂	β	H	γ	Proteins g%	Albumin g%	Quot A/G	Ascites and Oedeme
norm.	10/23	481		10. 6 50	457	12,4	18 4	191	0	4 4	5 07	2 32	0,84	(+)
norm.	9/25	—	—	14 7 50	478	12 9	21,3	13 4	0	4 6	4 58	2 19	0 91	(+)
norm.	8/21	362		10 8. 50	43 4	11 9	20 9	20 4	0	3 4	4 55	1 95	0 77	(+)
6 9	2/7	388		30. 8 50	36 9	13,5	23,8	19 6	0	6 2	3 85	1 42	0 58	0

WK ♂ 16 years † 3 9 50 S-Nr 976

FIGURE 12

biotic treatment a chronic condition de eloped which persisted for a period of years Hypoproteinemia with edema which followed could not be relieved in spite of blood and plasma infusions Four days after the last electrophoresis the pathologist found unexpectedly on post mortem examination subchronic liver atrophy Of great interest in this case was the fact that here is chronic liver atrophy without any typical changes in the blood protein pattern i e without the increase in the gamma globulin

Conclusion

In conclusion I should like to demonstrate how one can use electro phoretic blood protein patterns for the differential diagnosis of liver disease

From Table 3 we can make the following statements

Tl e re ersible acute li er d maze as found in virus hepatitis and infec tious mononucleosis is characterized by a moderate decrease in albumin content with a moderate increase in beta and gamma globulin The fibrinogen and sedimentation rates are increased

The irre ersible acute li er damage as found in liver atrophy shows a definite decrease in the albumin content with a reduction of the alpha

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The Use of Flocculation Tests in the Differential Diagnosis of Hepatitis

NOEL F MACLAGAN MD

(London England)

In common with the other members of the British delegation I should like first of all to express my thanks to the Henry Ford Hospital for inviting me to come here Also as another speaker has pointed out I should like to say that I did not choose the title of my communication While I shall do my best with it I feel that it will be difficult to tell the members of this symposium much that they do not already know about the use of flocculation tests in the differential diagnosis of hepatitis Perhaps the best thing I can do is to give you some of my personal experiences in this field and also an account of some new work that we have been doing at Westminster Hospital with the Takata Ara test

Figure 1 gives you a representative picture of the results one may expect with a typical flocculation test in jaundiced patients the jaundice being classified into three groups obstructive jaundice acute hepatitis and subacute and chronic hepatitis I think these results with the thymol turbidity test are similar to those found with many flocculation tests although there are marked differences between the various tests and one of the most important points is the more or less uniformly negative results obtained in cases of obstructive jaundice There is a high proportion of positive results in acute hepatitis and a rather lower proportion of positive results in the subacute and chronic cases

The case of acute hepatitis perhaps requires a little qualification since in this series it consisted mainly of acute epidemic infectious hepatitis and I think there are indications that the serum hepatitis probably gives a lower proportion of positive results This has not been very well documented except in the case of postarsphenamine jaundice which was probably syringe transmitted in which only about 60 per cent of positive results have been recorded[2] I think that Dr Neefe has found a similar low proportion of positive results in uncomplicated serum hepatitis

The uniformly negative results in obstructive jaundice have been difficult to explain They certainly do not indicate the absence of liver damage

In *liver abscess* we find a decrease in the albumin with a sharp increase in the alpha globulin as in infection with necrosis

In liver congestion due to *cardiac insufficiency* the electrophoretic pattern shows only slight changes so that a slight degree of liver damage may be assumed on the basis of the blood protein examination

The time allotted prevents me from giving further examples but I hope that I have demonstrated with sufficient clarity how the changes in the electrophoretic blood protein pattern fit our current classification of liver diseases

To conclude I should mention that the electrophoretic pattern is pathognomonic in only a few diseases such as plasmocytoma nephrosis and agammaglobulinemia The characteristic changes found in the blood protein pattern are not specific for liver disease but they permit conclusions as to diagnosis and prognosis

TABLE 1

EFFECT OF ADDING URINE MUCOPROTEINS TO TWO SERUMS GIVING POSITIVE FLOCCULATION REACTIONS

	Amount of Urine Mucoprotein Added mg /100 ml	*Flocculation Test*				
		Thymol Turb	*Thymol Floc*	*Colloidal Gold*	*ZnSO*	$(NH_4)_2SO_4$
Infectious Hepatitis Serum	0	8	4+	4+	9	3
(95 mg/100 ml) Fraction A_2	100	6	2+	2+	8	2.5
Added	500	2	0	0	6	2
Rheumatoid Arthritis Serum	0	5	2+	3+	8	6
(190 mg/100 ml) Fraction A_3	110	4	1+	1+	8	6
Added	586	2	0	0	6	4

were obtained with a urine mucoprotein fraction, serum mucoproteins react similarly.

It is valuable to know when these flocculation tests become positive in hepatitis. Undoubtedly the best data are by Neefe and Reinhold (1946) who were able to study a group of patients with experimental infectious hepatitis in whom they could follow all the tests from the beginning of the disease in each case. They found that the flocculation tests became positive around the fourth to the seventh day of the disease. Therefore in the early incubation stage of hepatitis one should not rely on these tests. They may be negative then and may become positive later. On the other hand Neefe and Reinhold showed that in the recovery stage of hepatitis the flocculation tests remained positive as a rule longer than any other of the tests used, and on the average the thymol flocculation test remained positive for the longest time. It was usually still positive some 70 or 80 days after the onset of the hepatitis. In recovering hepatitis, therefore, the flocculation tests seem to have a special significance.

The choice of flocculation tests depends to some extent on personal preference but Figure gives some data which may help in reaching a decision. This shows some typical results on a group of patients suffering from hepatitis, obstructive jaundice, and miscellaneous disorders; this last group was somewhat inflated by a large proportion of cases of rheumatoid arthritis which were being investigated at that time. Rheumatoid

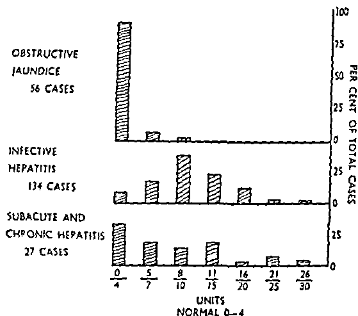


FIGURE 1 Thymol turbidity test in 217 cases of jaundice[3]

in obstructive jaundice nor the absence of a rise in gamma globulin in this condition which frequently occurs in cases complicated by cholangitis These considerations have suggested to many workers the presence of some inhibitory substance in the serum in obstructive jaundice Dr Ducci was first to show that the serums from obstructive jaundice did have an inhibitory effect We have added a little to this recently in some studies on the urine and serum mucoproteins which were carried out in another connection[5] We found that the patients with high serum mucoproteins on the whole had negative flocculation tests and the cases with positive flocculation tests on the whole had low serum mucoproteins This relationship suggested the experiment of adding mucoprotein to serums with positive flocculation tests which gave the results shown in Table 1

Two typical serums were used one from a case of infective hepatitis and one from a case of rheumatoid arthritis On adding pathologically possible amounts of mucoprotein it can be seen from Table 1 that with thymol and colloidal gold the results were reduced to almost zero while much less effect was produced on the zinc sulfate and ammonium sulfate test These findings give a possible explanation of the negative results in obstructive jaundice since the serum mucoprotein is known to be increased in this condition Incidentally although the results shown

It is however important to make the point that in the intermediate area here you find all types of jaundice Therefore if one finds an alka line phosphatase between 15 and 35 units and a negative thymol floccula tion test the results are not of much use in differential diagnosis One fifth of the cases fell into this equivocal group

In the remaining few minutes I would like to give you a preliminary report on some work that we have been doing on the Takata Ara test As Dr Voilier has mentioned this is a very popular test in Europe and Dr Angelo Bendandi from Italy who was working with me this year was particularly anxious to do some work on this test We felt first of all it should be brought up to date by using a phosphate buffer to standardize the pH and under these conditions we were surprised to find that the ionic strength needed to be about 1 0 or higher to get a good differentia tion between normal and hepatitis serum Under these conditions there is an optimal distinction between the normal and hepatitis serum at about pH 12 3 and the amount of mercuric chloride required varies directly with the pH

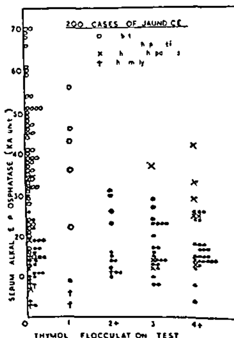


FIGURE 3 Results with thymol flocculation test and serum alkaline phosphatase in 200 cases of jaundice[5]

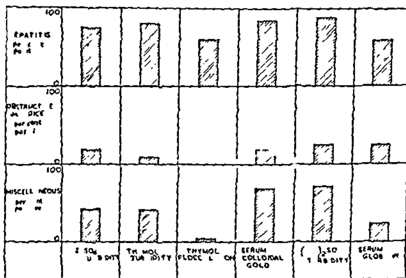


FIGURE 2 Comparison of flocculation tests in jaundice and other conditions[4]

arthritis is one of those diseases which also gives positive flocculation tests in quite a proportion of cases and this fact serves to emphasize the nonspecific nature of the tests

It can be seen from Figure 2 that the zinc sulfate and the thymol turbidity are not very far apart and the serum colloidal gold is not very dissimilar but gives a rather higher proportion of nonspecific results There is however a considerable difference if one uses thymol flocculation instead of thymol turbidity This was first pointed out by Dr Neefe and Dr Reinhold and I think it is an important contribution to the study of jaundice *In the differential diagnosis of jaundice it can be seen that* the thymol flocculation test gives a much sharper differentiation than does the thymol turbidity or any other tests shown

The flocculation tests are particularly suitable for combination with a serum alkaline phosphatase because these two tests go in opposite directions The serum alkaline phosphatase is nearly always high in obstructive jaundice whereas the flocculation tests are usually negative in this condition By combining the two one gets a picture rather like a two way chromatogram In Figure 3 are plotted the results of the two tests *in 200 pedigree cases of jaundice The top left hand area shows that* the cases with high alkaline phosphatases (over 35 units) and negative thymol flocculation tests were uniformly obstructive *All those with* strongly thymol flocculation were nonobstructive and all with phosphatases below 15 King Armstrong units were nonobstructive This accounts for four fifths of the cases

of hepatic cirrhosis many provided by the kindness of Dr Sherlock o of these gave positive results and 18 were classed as strongly positive (more than 6 units)

All the other cases with liver pathology tested also gave positive results and these were also seen in a proportion of the miscellaneous group of cases shown in Table 3 It appears therefore that this new test would not be useful in differential diagnosis These nonhepatic positive results were partly due to the inclusion of a large group of cases of ulcerative colitis a disease in which many workers have described liver damage The mercuric chloride test shows some correlation with the zinc sulfate test but appears to be somewhat more sensitive since only 16 of the 23 cases of cirrhosis referred to above were positive with zinc sulfate

In conclusion the new mercuric chloride turbidity test although of limited value in the differential diagnosis of jaundice appears to be a rather sensitive detector for the type of serum protein disturbance which occurs in hepatic cirrhosis The same disturbance can however occur in other diseases and the nonspecific nature of the test must be recognized

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The next step was to investigate the use of the different salts under these conditions Of a number of common metals tried differentiation between normal and hepatitis serum was only seen with mercuric chloride and silver nitrate This suggests that the mechanism of the test depends upon the unstable hydroxide of these two metals which are peculiar in that they do not possess a hydroxide It seems that under these conditions only those metals with unstable hydroxides react with the protein complex found in the serum in liver disease On the basis of these experiments we adopted the technique shown in Table which gives results of from 0–4 turbidity units in a group of normal subjects

Table 3 gives an over all picture of the results obtained with the new mercuric chloride turbidity test As with the old Takata reaction we were particularly successful with hepatic cirrhosis There were 23 cases

TABLE 2

THE MERCURIC CHLORIDE TURBIDITY TEST

0 05 ml serum 3 ml buffer x ml M/10 $HgCl_2$

*p*H	11 3	11 7	12 3	12 9	13 3
x	0 1	0 15	0 5	0 4	0 5
Normal	4	3	2	5	5
Hepatitis	7 F	7 F	8 F	6	5

F = rapid flocculation

TABLE 3

MERCURIC CHLORIDE TURBIDITY TEST IN 268 CASES

Condition	*No of Cases*	*No Positive*	*No Strongly Positive (Over 6 Units)*
Normal	37	0	0
Gastrointestinal diseases			
Ulcerative colitis	60	20	11
Miscellaneous	22	7	1
Liver diseases			
Cirrhosis (portal cirrhosis 10 primary biliary cirrhosis 4 secondary biliary cirrhosis 2 posthepatitis 4 ulcerative colitis + cirrhosis 3)	23	20	18
Viral hepatitis	3	3	3
Asymptomatic posthepatitis	4	3	2
Obstructive jaundice	3	3	1
Miscellaneous (amoebic hepatitis 1 hepatomegaly 3 jaundice 4)	8	3	3
Miscellaneous positive	30	30	14
Miscellaneous negative	78	0	0

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The Clinical Significance of Alterations in Serum Transaminases in Hepatitis

FELIX WRÓBLEWSKI M D
(New York New York)

Alterations in the enzymatic activity of two serum transaminases glutamic oxaloacetic and glutamic pyruvic have been shown to be clinically significant in the differential diagnosis of hepatic disease states[1] It is the purpose of this paper to review and extend the observations made in regard to these two serum enzymes as they apply particularly to the diagnosis and differential diagnosis of hepatitis

Transamination is a chemical reaction in which there is an exchange of the alpha amino group of an amino acid for the keto group of an alpha keto acid with the resulting synthesis of a second alpha amino acid and a new alpha keto acid This type of chemical conversion was first described in 1937 and was postulated to be a reaction which occurred with any amino acid and alpha ketoglutarate or oxaloacetate using pigeon breast muscle as a source of transaminase[2] Using a coenzyme pyridoxal phosphate it was shown that muscle homogenates contained transaminase capable of catalyzing the transfer of alpha amino groups from 25 different amino acids including aspartic and alanine and that this enzyme was present in pig heart liver and kidney tissue homogenates[3] Subsequent observations indicated that the organ distribution of transaminase activity is species specific and in man glutamic oxaloacetic transaminase (GO T) is present in heart liver skeletal muscle and kidney and glutamic pyruvic transaminase (GP T) is present in liver kidney heart and skeletal muscle in decreasing order respectively[4] (Figure 1) The presence of GO T and GP T activity was demonstrated in whole blood serum and plasma and the range of normal serum activity was delineated[5] Concomitantly elevations of serum glutamic oxaloacetic transaminase (SGO T) and serum glutamic pyruvic (SGP T) transaminase of patients with hepatic and other disease states were observed[5] The separation of the two serum enzymes was accomplished by fractional electrophoresis of serum protein in order to establish that the enzymatic activities in serum represent two distinct and separable proteins rather than the same enzyme acting on different substrates[6] (Figure)

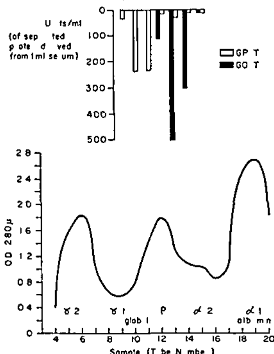
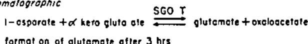
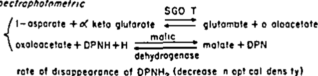


FIGURE 2 Separation of glutam c xal acetic an l glutamic pyruvic trans amina e by fracti nal electrophoresis of the serum from a 60-year old male w th acute homol gous serum hepatitis and intrahepatic meta ta e carcinoma SGP T act ity is confined primarily to Fractions 9 to (gamma gl l ulin) SCO T activity i primar ly dem n trable in Fractions 12 to 14 (beta globul n)





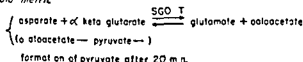


FIGURE 3

NORMAL HUMAN TISSUE (u/gm of wet tissue)	GO-T*	GP-T**
HEART	155,500	7,130
LIVER	142,400	43,800
SKELETAL MUSCLE	99,300	4,750
KIDNEY	90 900	19,300
PANCREAS	28,300	1,950
SPLEEN	13,600	1,210
LUNG	10,000	668
SERUM	20	15

* glutamic-oxaloacetic transaminase activity
** glutamic-pyruvic transaminase activity

FIGURE 1 Distribution of glutamic-oxaloacetic and glutamic pyruvic transaminase in normal adult tissue homogenates

METHODS OF MEASUREMENT OF SGO T AND SGP T ACTIVITY

Chromatographic spectrophotometric and colorimetric techniques have been used to measure SGO T[5 7 8] (Figure 3) and SGP T[4 5 9] (Figure 4) Quantitative paper chromatographic analysis permits the measurement of SGO T and SGP T by estimating the amount of glutamic acid formed during a finite incubation period Using this method the mean SGO T and SGP T activity expressed as micromoles per milliliter per hour of glutamate formed is 0 6 ± 0 191 and 0 5 5 ± 0 146 respectively[5] The chromatographic technique although accurate is cumbersome and lengthy

Spectrophotometrically SGO T and SGP T activity are assayed by employing a double enzyme system Glutamic oxaloacetic and glutamic pyruvic transamination are coupled to the enzymatic oxidation of reduced diphosphopyridine nucleotide using malic dehydrogenase (Figure 3) in the former and lactic dehydrogenase (Figure 4) in the latter transaminase determination The spectrophotometric techniques measure SGO T and SGP-T by estimating the rate of the enzymatic reaction rather than any one of the end products of the transamination Spectrophotometrically SGO T and SGP T activity are expressed as units per milliliter of serum per minute One unit equals a decrease in optical density of 0 001 millimicron under the conditions specified in the literature[4 7] The mean SGO T activity of normal adult serums is 1 + − 6 8 with the range of normal 5 to 40 units The mean SGP T activity of normal adult serums is 16 + − 9 with the range of normal 5 to 35 units[4] These tech

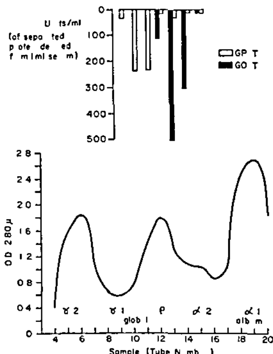
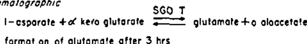
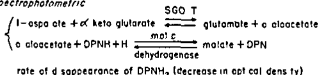


FIGURE 2 Separation of glutamic oxaloacet c and glutamic pyruvic trans am nase by fracti nal electrophoresis of the serum from a 60 year-old male with acute homol gous serum hepatitis and intrahepatic metasta c carcinoma SGP T activity is confined primarily to Fractions 9 to 11 (gamma gl bul n) SGO T activity is primarily dem n trable in Fractions 12 to 14 (beta globulin)

SGO-TRANSAMINASE ASSAY METHODS





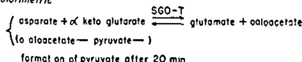


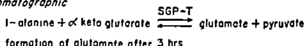
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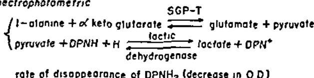
niques are simple rapid and accurate but require equipment and reagents which may not be generally available

The colorimetric methods estimate SGO T and SGP T by measuring the amount of pyruvic acid formed under standard incubation conditions[8, 9] Pyruvic acid is formed directly during the glutamic pyruvic transamination and indirectly by the conversion of oxaloacetic acid to pyruvic acid following glutamic oxaloacetic transamination These methods are simple and require no enzymes as reagents but are somewhat less accurate and less sensitive than the other methods Colorimetrically SGO T and SGP T activities are expressed as units per milliliter of serum One unit equals the formation of one microgram of pyruvate under the conditions specified The mean SGO T activity of normal adult serums is 16.4 + − 8.4 units with a range of normal 4 to 40 units[8] The mean SGP T activity of normal adult serums is 20 + − 11.5 with a range of normal 1 to 45 units[9] Transaminase activity has been found in all human and animal serums tested both in the normal and diseased state

The stability of these serum enzymes is such as to facilitate their clinical usefulness Specifically freezing and lyophilization of serum fail to influence SGO T or SGP T activity Serums and heparinized plasma have equivalent activities and storing serum at room temperature for 24 hours or at 4 C for 5 days does not significantly alter SGO T or SGP T activity The fasting state does not influence SGO T and SGP T levels within the normal range and the variations from day to day in SGO T in the same normal adult are insignificant[4, 5] The mechanism for excretory and/or secretory handling of SGO T and SGP T is unknown, but the

SGP-TRANSAMINASE ASSAY METHODS





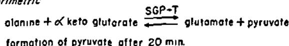
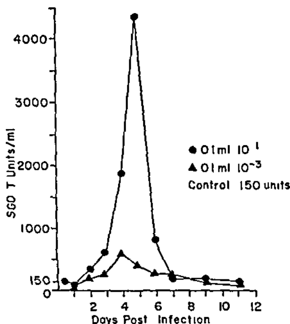


FIGURE 4

presence of these enzymes in small amounts in urine and in appreciable amounts in bile suggests that renal and biliary routes may contribute in this regard[10] It is pertinent however that oliguria or azotemia do not in themselves appear to influence the height of SGO T or SGP T within the normal or abnormal range of activity[1]

Alterations of Serum Transaminase in Experimentally Produced Pathologic Hepatic States

Experimentally produced virus hepatitis in mice has been associated with increase in SGO T[11] and SGP T[1] activity A relationship appears to exist between the rise in SGO T activity and the size of the virus inoculum (Figure 5) the blood virus titer (Figure 6) and the degree of liver necrosis[11] The serial alterations of SGO T in virus hepatitis in mice are paralleled by changes in SGP T activity which is proportionately increased to a greater extent above the normal range for mice than is SGO T activity[1] (Figure 7) The injury of hepatic tissue accompanying partial hepatectomy in mice is associated with elevations in serum transaminase[11] When hepatocellular injury is produced in rats with carbon



SGO T Activity in mice following intraperitoneal inoculation of 0.1 ml 10^{-1} and 0.1 ml 10^{-3} dilution of hepatitus virus

Figure 5

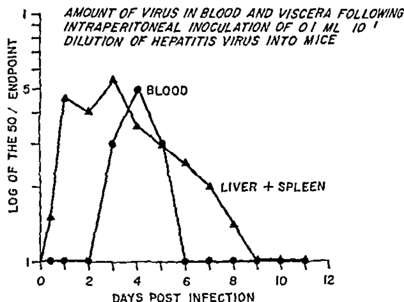
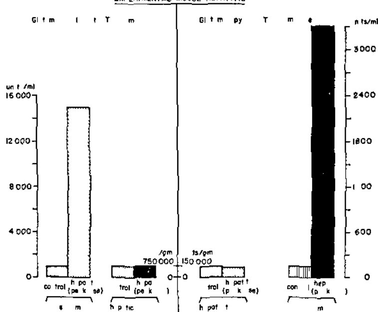


FIGURE 6 The serial alterations of the blood virus titer approximately parallel the SGO T changes

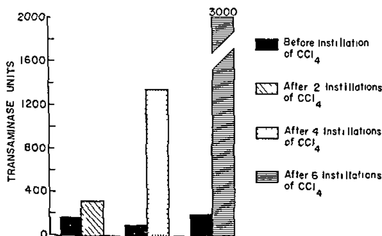
tetrachloride it has been observed that SGO T alterations serve as a sensitive index of hepatocellular injury the height and duration of increased SGO T activity has been found to be proportional to the amount of toxin administered and to the extent of liver cell damage [13] (Figure 8) It has been reported in experimental carbon tetrachloride toxic hepatitis that hepatic tissue glutamic oxaloacetic transaminase is not decreased even when SGO T is concomitantly and markedly elevated [14] The same observation has been made in regard to hepatic tissue glutamic oxaloacetic and glutamic pyruvic transaminase during the course of virus hepatitis in mice at the time that SGO T and SGP T activities were impressively elevated [1] Cirrhosis and hepatic tumors experimentally produced in rats using butter yellow have been shown to be accompanied by elevated SGO T activity [1, 1] Common duct occlusion experimentally produced in dogs has resulted in elevations in SGO T activity which returned to normal within a week following the relief of biliary tract obstruction [17]

ALTERATIONS IN SERUM TRANSAMINASE IN ACUTE HEPATITIS

Acute hepatic disease has been noted to be associated with rises in SGO T and SGP T activity [2 4 18] In most instances the quantitative and serial changes in these two serum enzymes are sufficiently characteristic of the various types of liver disease to assist in diagnostic differentiation [18] The largest elevations in SGO T and SGP T have been observed in acute toxic hepatitis due to carbon tetrachloride and in patients with



Fi URE 7 Compari on of SGO T SCP T and hepatic tissue GO T GP T during the c urs of vir hepat t s in mice



Fi URE 8 Comp ison of peal elev t n n SGO T w th arving doses of cart n tetrachl ride durir g th o se of experimentally prod ced toxic hepat ti in rat

acute infectious and/or homologous serum hepatitis [1, 18] The rise in both serum transaminases following exposure to carbon tetrachloride occurs within 24 hours and has reached levels as high as 7 000 units With cessation of exposure to the toxin SGO T and SGP T precipitously fall toward normal The alterations in SGP T parallel those seen in SGO T activity but are usually greater in the former than in the latter Toxic hepatitis due to thorazine [19] salicylates [0] cincophen [0] azoserine [0] pyrazinamide [1] (Figure 9) and other agents is usually associated with smaller elevations of serum transaminases than have been observed in carbon tetrachloride toxic hepatitis Continued increments in the serum enzymes are observed with continued administration of these drugs when they prove to be hepatotoxic discontinuance of the hepatotoxic agent results in a rapid fall toward normal of serum transaminases

Acute liver cell injury as seen in acute infectious and homologous serum hepatitis results in impressive increments in both serum transaminases Although the changes in the activity of the two serum enzymes parallel each other the rise of SGP-T usually exceeds that of SGO T activity (Figure 10 Figure 11) It appears that the rise in serum transami

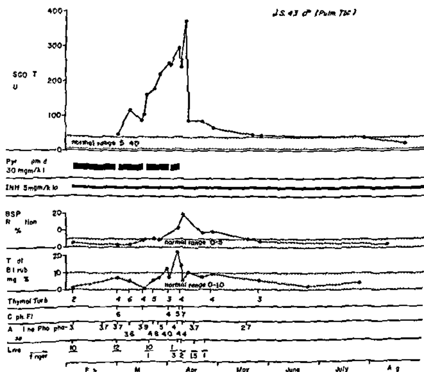


FIGURE 9 Alterations in SGO T activity and liver function tests during the course of toxic hepatitis due to pyrazinamide

nases in virus and homologous serum hepatitis begins during the prodromal phase of the disease (Figure 12) It reaches a peak elevation of 10 to 100 times the normal serum activity at the time the patients are the sickest as adjudged by fever malaise anorexia nausea vomiting and hepatic tenderness With subjective and objective evidence of improvement a fall in both serum transaminases toward normal occurs

The natural uncomplicated course of infectious hepatitis is associated with a gradual rise in activity of both serum transaminases to a peak followed by a gradual decrease in serum enzyme activity toward the normal range during the recovery phase When complications occur during the course of hepatitis the added stress appears to influence the hepatitis and this is reflected in a secondary superimposed rise in SGO T and SGP T activity Ambulation during recovery from infectious hepatitis is sometimes associated with a small rise in serum transaminase If the rise following ambulation is 50 or more units return to rest discipline would appear advisable and SGO T and SGP T usually resume the return toward normal Relapses of infectious and homologous serum hepatitis are associated with secondary rises in SGO T and SGP T activity (Figure 13) Unresolving hepatitis is associated with persistently elevated serum transaminases at the time the serial alterations would be expected to

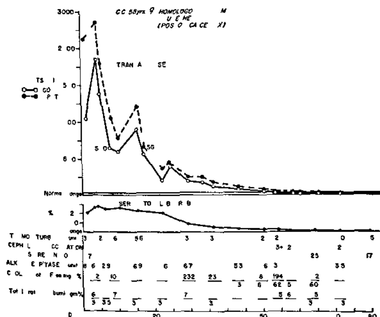


FIGURE 10 Serial alterations SGO T and SGP T activities and tests of liver function during the course of homologous serum hepatitis

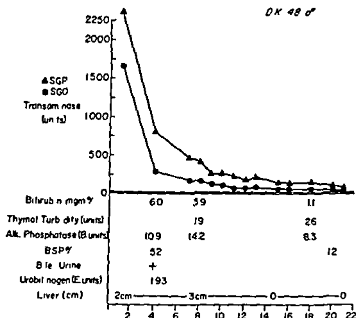


FIGURE 11 Serial alterati ns in SGO T and SCP T activities and tests of liver function during the co urse of infecti ous hepatitis

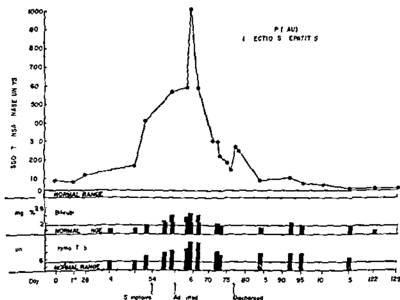


FIGURE 1 Serial alterations n SGO T activity and tests of l ver function during the prodromal and s mptomatic phase of nfectious hepatitis.

return toward normal The failure of SGO T and SGP T activity to return to the normal range suggests the development of chronic hepatitis and/or postinfectious cirrhosis The serial alterations of SGO T and SGP T *in the course of acute infectious hepatitis follow a characteristic* pattern deviations from this usual course of enzyme alterations suggest associated complications relapses and/or chronicity of the hepatic infection It appears that the serial and quantitative changes in SGO T and SGP T during the course of hepatitis reflect the clinical state of the patient more accurately than conventional liver function tests In this regard SGO T and SGP T are thought not to reflect liver cell function *per se* but rather to represent the reaction to acute liver cell injury Accordingly the serum transaminase alterations are not necessarily found to correlate with conventionally employed tests of liver function changes in serum transaminase do not appear to be an index of liver cell function The sensitivity of serum transaminase as a reflection of liver cell injury may account for the observation that in acute hepatitis SGO T and SGP T are elevated in the prodromal and clinical phase of the disease at a time when tests of liver cell function are as yet unaltered (Figure 1 and Figure 14)

Observations during the course of an epidemic of acute infectious hepatitis in a closed environment indicated that elevations in serum transaminase more sensitively reflect subclinical hepatitis than do conventional liver function tests [1] Using both liver function tests and elevations

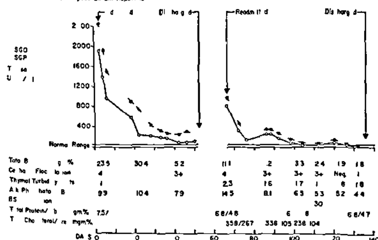


FIGURE 13 Alterations in SGO T and SGP T activities and tests of liver function during the course of homologous serum hepatitis in a patient who incurred a relapse

of serum transaminase as criteria individuals examined during the course of an institutional epidemic of acute infectious hepatitis fell into 5 groups (a) asymptomatic individuals with normal SGO T serum bilirubin and thymol turbidity (b) asymptomatic individuals with transiently abnormal SGO T (up to 100 units) and with normal serum bilirubin and thymol turbidity (c) asymptomatic individuals with abnormal SGO T (up to 350 units) normal serum bilirubin and abnormal thymol turbidity (d) symptomatic individuals with abnormal SGO T (up to 350 units) hyperbilirubinemia and abnormal thymol turbidity (e) symptomatic individuals with clinical icteric hepatitis The individuals in the groups above were categorized respectively as (a) normals (b) contacts (c) nonicteric hepatitis individuals and (d) subicteric hepatitis individuals. The final group of inmates consisted of those individuals who developed icteric clinical infectious hepatitis for which they required hospitalization Figure 15 shows the SGO T alterations serum bilirubin and thymol turbidity in individuals in 4 of the 5 categories a normal individual typically shows normal values of SGO T serum bilirubin and thymol turbidity

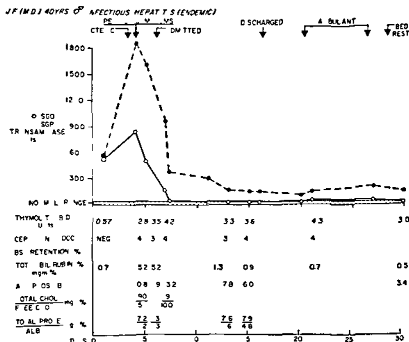


FIGURE 14 Normal liver function tests are recorded during the prodromal phase of infectious hepatitis at a time in the course of the disease when serum transaminase activities are elevated above the normal range

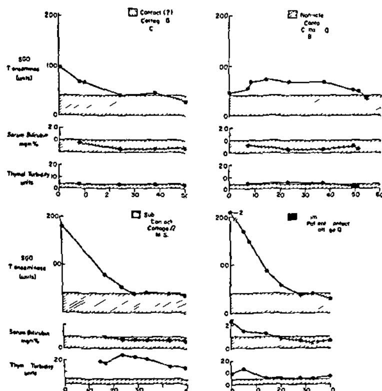


FIGURE 15 Comparison of serum bilirubin, thymol turbidity and SGO T in contacts, nonicteric, subicteric and minimally icteric hepatitis individuals

These and other epidemiologic observations suggest that asymptomatic nonicteric and subicteric hepatitis individuals as evidenced by increased serum transaminase may possibly communicate the disease without themselves being recognized as having clinical hepatitis. Whether alterations in SGO T in the serum of blood donors may contribute to the detection of those individuals who serve to transmit homologous serum hepatitis is presently under study.

Infectious mononucleosis is usually accompanied by normal SGO T and SGP T activity. However, when this malady is complicated by hepatitis there is a rise in SGO T and SGP T at a time when liver function may be normal or inconclusively affected as measured by conventional tests[18] (Figure 16). The severity of the hepatitis accompanying infectious mononucleosis appears to be related quantitatively to the peak rise in SGO T and SGP T and the latter is greater than the former throughout the course of the elevated serum transaminase activity.

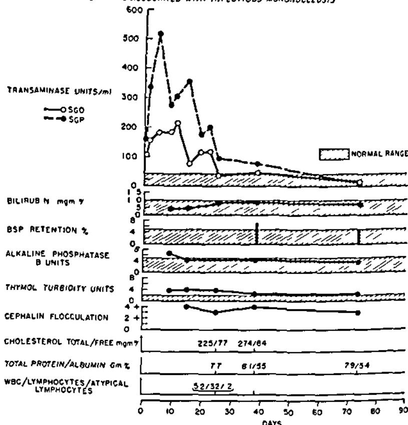


FIGURE 16 Serial alterations in SGO T and SGP T activities and tests of liver function during the course of acute hepatitis complicating infectious mononucleosis

Alterations in Serum Transaminase in Other Disease States

Active Laennec's cirrhosis and biliary cirrhosis are associated with elevations in SGO T in the range of 50 to 00 units[1] When SGP T is concomitantly increased it is of lower activity than SGO T[18] (Figure 17) Cirrhosis complicated by acute hepatitis has been noted to present serum transaminase alterations characteristic of acute hepatitis but superimposed on the serum enzyme elevations due to hepatic cirrhosis (Figure 18) Accordingly serum transaminase may be helpful in determining whether one is dealing with sudden hepatic decompensation associated with cirrhosis or superimposed acute hepatitis in the cirrhotic patient With sudden hepatic decomposition without hepatitis no superimposed rise in serum transaminase has been observed

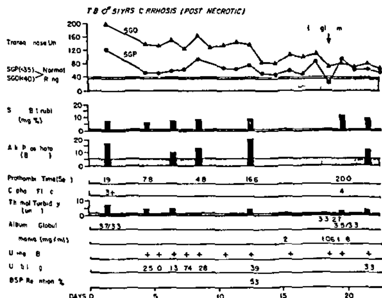


FIGURE 17 Serial alterations in SGO T and SGP T activities and tests of liver function during the course of active postnecrotic cirrhosis

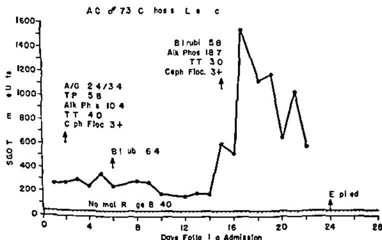


FIGURE 18 Serial alterations in SGO T and SGP T activities and tests of liver function during the course of [illegible] cirrhosis with superimposed hepatitis during the final 8 days of the patient's life

Extrahepatic biliary obstructive jaundice is characterized by increments in transaminase activity from 40 to 00 SGO T units and 50 to 300 SGP T units Although both enzymes are altered in the same direction the SGP-T activity usually exceeds the corresponding SGO T activity in acute extrahepatic biliary obstruction[18] (Figure 19) The serum enzyme activities return to normal usually within a week after relief of the biliary obstruction

Serum transaminase activity is as sensitive an index of primary and metastatic cancerous involvement of the liver as the alkaline phosphatase but it is unaffected by the presence of active metastatic bone cancer This property provides a means of distinguishing between elevated alkaline phosphatase due to liver and that due to bone disease When both the SGO T and alkaline phosphatase are elevated liver disease can be assured to be present but when the alkaline phosphatase is increased and the transaminase is normal the elevated alkaline phosphatase is due primarily to bone disease The degree of increased transaminase activity seen in metastatic cancer to the liver is roughly proportional to the amount of liver cell injury resulting from tumor growth When SGP T is concomitantly increased along with elevated SGO T in metastatic liver disease it is of lower activity than SGO T[18] (Figure 20)

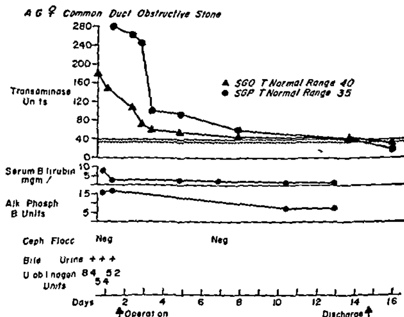
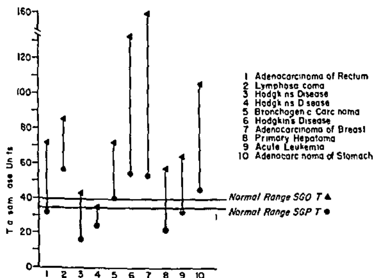


FIGURE 19 Serial alterations in SGO T and SGP T activities and tests of liver function during the course of extrahepatic common duct obstruction due to stone



COMPARISON OF SGP T AND SGO T IN PATIENTS WITH LIVER INVOLVEMENT BY CARCINOMA LYMPHOMA AND LEUKEMIA

FIGURE 20

Serum transaminase levels are not elevated in patients with infectious neoplastic degenerative reactive allergic or congenital disease or pregnancy states unless evidence of acute damage to the liver heart or skeletal muscle is present Surgical trauma to skeletal muscle acute myocardial infarction and any process associated with acute injury to skeletal muscle or heart muscle may result in increased serum transaminase activity The jaundice associated with hemolytic processes is not usually associated with significant alterations in serum transaminase activity The alterations in serum transaminases in nonhepatic states present little or no confusion diagnostically in as much as they appear in different clinical settings and are reflected by alterations in transaminase of appreciably different magnitude and serial change In all of these clinical settings whenever there is a rise in SGO T there is consistently an appreciably smaller elevation of SGP T activity

Summary and Conclusions

SGO T and SGP T alterations are sensitive and roughly proportional indices of hepatocellular injury during acute hepatitis of various etiologic types The elevations of SGP T activity during the course of acute hepatitis exceed those observed for SGO T Acute and active chronic hepatic disease are associated with quantitative and serial elevations of SGO T and/or SGP T which are sufficiently characteristic to permit

diagnostic differentiation SGO T alterations more sensitively reflect active chronic hepatic disease such as active cirrhosis and primary and metastatic cancer of the liver SGP T appears to be even more sensitive than SGO T in reflecting acute hepatocellular injury associated with acute hepatitis and acute extrahepatic biliary obstruction By the simultaneous measurement of SGO T and SGP T activity it appears possible in most cases to differentiate acute from active chronic liver cell injury The quantitative and serial measurements of both enzymes permit accurate differential diagnosis of many clinical types of hepatic disease

The subjective and objective clinical course of patients with acute hepatitis appears to be accurately reflected in serial SGO T and SGP T alterations Relapses exacerbations chronicity premature ambulations and unrelated superimposed complications in patients with acute hepatitis are usually evidenced in variations from the usual pattern of serial serum transaminase changes The serum transaminase changes observed in the prodromal phase of hepatitis and in individuals with nonicteric and/or subicteric types of acute hepatitis permit a better understanding of the epidemiologic course of this disease and facilitate the diagnosis and thereby the management of individuals with subclinical and otherwise *unrecognized hepatitis*

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Bile Pigments of Serum in Disease of Liver

JESSE L BOLLMAN MD
(Rochester Minnesota)

Recent advances in methods of separating and identifying biliary pigments in bile serum and urine clearly indicate that present concepts of the metabolism of bile pigment are inadequate or incorrect These methods provide a means for revaluating the physiology of formation and excretion of bile pigment and also the aberrations produced by pathologic processes involving the liver

Cole and Lathe[1] using reversed phase chromatography found that the elution of bile pigments from a column of siliconed infusorial earth with methanol clearly separated two types of bilirubin Further studies of elution by means of a system of *n* butanol and phosphate buffer clearly separated two pigments from the more polar rapidly moving fraction Billing s[3] modification of this method removes three distinct bile pigments from the column The most polar pigment moves rapidly on the column and is designated pigment II by Cole Lathe and Billing[4] Pigment II constitutes about 75 per cent of the bile pigment of normal human bile and is not detectable in blood of normal persons but it is often present in the blood and urine when nonhemolytic types of jaundice occur Diazotization of pigment II gives pigment b which also moves rapidly from a column when eluted with *n* butanol in phosphate buffer Further study and that of Overbeek Vink and Deenstra indicated that the tetrapyrrolic bilirubin molecule splits to form two diazotized dipyrroles Those from pigment II were clearly different from the two dipyrroles from diazotized bilirubin which were designated as pigment a and moved very slowly on the same column system which moved pigment b rapidly Billing and Lathe[6] and Schmid[7] recently have established that pigment II is an ester diglucuronide of bilirubin This pigment II is water soluble and when isolated in pure form or reconstituted in serum it reacts immediately with the van den Bergh reagent The diglucuronide was readily hydrolyzed to form bilirubin which was insoluble in water and reacted only indirectly with the van den Bergh reagent The ready hydrolysis of the water soluble pigment II of bile accounts for the fact that chemical preparations of bile pigment from bile yield insoluble bilirubin from the soluble pigment of bile It is also the probable basis for the formation of bilirubin concretions in diseased biliary ducts

The bile pigment which moved less rapidly on the Billing s column

ments are formed from hemoglobin as it is degraded by the reticuloendothelial cells of the bone marrow and spleen In the absence of the liver this bile pigment accumulates in the blood [8] and gives an indirect or delayed reaction to the van den Bergh s reagent When this bile pigment is excreted in the bile it gives a prompt direct van den Bergh reaction If biliary excretion is prevented by obstruction of the biliary ducts the bile pigment retained in the blood reacts directly with the van den Bergh reagent If the liver is removed subsequent to the development of obstructive jaundice the direct reacting serum bilirubin remains subsequently at the same level but the total bilirubin of the blood continues to increase because of the additions of indirect reacting bile pigment from the reticuloendothelial system [9]

It was noted however that the indirect reacting bilirubin of the liver less animal was not exactly like the indirect reacting bilirubin of the blood of normal human beings or of those having hemolytic icterus As the amount of bilirubin in the serum increased after hepatectomy the indirect van den Bergh reaction became more rapid and bilirubin appeared in the urine Neither of these findings was noted in human beings when only indirect reacting bilirubin was present in the blood The bilirubin in urine gave a direct reaction to the van den Bergh reagent. Clinical appraisal of the amounts of direct and indirect reacting bilirubin in the serum of icteric human beings [10] showed that the indirect form was found in the presence of hemolytic types of jaundice but that the direct reaction was present in obstructive jaundice and also during hepatocellular jaundice It was soon found however that the blood of many icteric human beings contained both types of bilirubin in varying proportions Attempts to classify jaundice according to the relative amounts of indirect and direct reacting bilirubin in serum have been only partially successful and often fail when both types are present

A revaluation of knowledge concerning the metabolism of bile pigment is necessary The finding of pigment I raises the question of its metabolic significance and also the question of whether its unrecognized presence in previous studies has led to erroneous conclusions with regard to the metabolic aspects of pigment II and bilirubin

I have confirmed Cole and Lathe s observations that the pigment in gallbladder bile of human beings is about 75 per cent pigment II and 25 per cent pigment I and contains only traces of bilirubin I found similar concentrations of bile pigment in gallbladder bile from normal dogs Hepatic bile from dogs with biliary fistulas also showed similar proportions of pigment I and II although the concentration of total pigment was less than that of gallbladder bile The pigment in bile obtained from biliary fistulas of rats was 80 to 90 per cent pigment II and 10 to o per cent pigment I These figures indicate that the liver secretes both pigment

was designated as pigment I This pigment also gives a direct' reaction to the van den Bergh reagent that is it reacts in an aqueous solution without the presence of organic solvents Pigment I is soluble in water but is more soluble in chloroform and is readily extractable from aqueous solutions by chloroform whereas pigment II remains in the aqueous phase Pigment I constitutes about 25 per cent of the bile pigment of normal bile and is not detectable in normal blood but it is often present in the blood of jaundiced persons Diazotized pigment I when eluted from a column with *n* butanol in phosphate buffer forms two bands One moves rapidly and is similar to pigment b (diazotized pigment II) and the other moves slowly and appears identical to pigment a (diazotized bilirubin) This observation is the basis for the suggestion that pigment I is a monoglucuronide of bilirubin which forms two dipyrroles on diazotization One dipyrrole pigment a is similar to that from bilirubin and one dipyrrole pigment b is similar to that from the diglucuronide pigment II

Bilirubin was eluted slowly from the columns of Cole Lathe and Billing It is insoluble in water but is a soluble salt in alkaline solutions and is soluble in chloroform Only traces of bilirubin are found in fresh normal bile and it is present in normal human blood It is not excreted in the urine of jaundiced persons in whom the amount of bilirubin (indirect reacting) is often found to be above the normal level Diazotized bilirubin (pigment a) moves slowly from a column eluted with *n* butanol in phosphate buffer

Since the isolation and identification of these three biliary pigments has been accomplished many of the controversies with regard to the nature of the van den Bergh reaction may be clarified Pigment I and pigment II are rapidly diazotized when in aqueous solution or serum whereas water insoluble bilirubin is diazotized only slowly in aqueous suspension The addition of alcohol or other organic solvents greatly increases the speed of this reaction probably by providing solution of bilirubin and much greater contact with the reagent The van den Bergh reaction however provides no differentiation between pigments I and II I have been unable to modify the van den Bergh reaction so that determination of the direct and indirect reacting pigment present corresponds to that found on column chromatography Good correlation may be obtained when most of the pigment is indirect or when most of the pigment is direct and *pigment II Pigment I in serum reacts directly to the* van den Bergh reagent at a slower rate than pigment II so that the reaction is not complete before considerable amounts of bilirubin may be diazotized

Concepts concerning the formation of bile pigment which were formed before recognition of the third bile pigment hold that bile pig

ments are formed from hemoglobin as it is degraded by the reticuloendothelial cells of the bone marrow and spleen In the absence of the liver this bile pigment accumulates in the blood [8] and gives an indirect or delayed reaction to the van den Bergh s reagent When this bile pigment is excreted in the bile it gives a prompt direct van den Bergh reaction If biliary excretion is prevented by obstruction of the biliary ducts the bile pigment retained in the blood reacts directly with the van den Bergh reagent If the liver is removed subsequent to the development of obstructive jaundice the direct reacting serum bilirubin remains subsequently at the same level but the total bilirubin of the blood continues to increase because of the additions of indirect reacting bile pigment from the reticuloendothelial system [9]

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II and pigment I in the bile I also studied gallbladder bile obtained at necropsy from two jaundiced persons who succumbed to liver disease Only 35 and 40 per cent of the bile pigment respectively were pigment II the remaining 65 and 60 per cent were pigment I These proportions of pigments I and II corresponded closely to those in the blood withdrawn from the same persons a few days before death

Study of retention icterus which was produced in dogs by ligation of the common bile duct disclosed that it was accompanied by the appearance of pigment II in the blood Only traces of bilirubin were present and the direct reacting pigment was 80 per cent pigment II and o per cent pigment I during the first week of obstructive jaundice Rats also were found to have mainly pigment II in their blood for the first few days after ligation of the biliary duct (pigment II 80 per cent and pigment I o to 5 per cent) During the second week of obstructive jaundice in the dog the concentration of pigment I in the blood began to increase and continued to increase subsequently The total bile pigments of the blood did not increase so that the increase of pigment I was associated with decrease of pigment II in the blood These findings indicate that early in retention of bile pigments II and I which are excreted in bile normally are retained in the blood and much of pigment I is converted to pigment II Continued biliary obstruction appears to produce sufficient damage to the liver so that less conversion of pigment I to pigment II occurs

Dogs becoming jaundiced after administration of toluenediamine were found to have an increase of pigment I pigment II and bilirubin in their blood The relative amounts of each varied from animal to animal and at different times during the course of the jaundice From 37 to 65 per cent of the direct reacting bile pigment of the blood appeared as pigment II

Repeated administration of ethionine produced jaundice in dogs In this form of icterus pigment I but not pigment II was found in the blood In my laboratory also we have produced jaundice in one rabbit and one rat by the administration of icterogenin a pentacyclictriterpene extracted from *Lippia rehmanni Pears*[11] During a period after the use of this drug pigment free bile may be excreted The pigment retained in the blood of the two animals which I have studied appeared only as pigment I

After complete removal of the liver from dogs the yellow color of bile pigment becomes apparent in the blood tissues and urine and the degree increases progressively The yellow may be observed visually in 6 to 10 hours after removal of the liver and after this lapse of time tests for bile pigment in the blood and urine are definitely positive About 4 hours after hepatectomy the bilirubin content of the serum has reached about 3 mg for each 100 ml of serum The van den Bergh reaction is more rapid than the typical indirect reaction but not as rapid as the typical direct reaction of obstructive jaundice Treatment of this serum

by the methanol method of Cole and Lathe definitely disclosed two pigments one which was rapidly eluted and another which was eluted more slowly The first pigment eluted had all of the characteristics of pigment I Elution from the column with butanol was slower than that of pigment II The diazotized pigment eluted from a column in the two bands which characterized pigment I The pigment found in the urine also appeared to be pigment I No pigment II could be found in either the blood or urine of the liverless dog In each dog of this series more of pigment I than bilirubin was found in the serum (Figure 1) No direct estimate of the total amount of each of the bile pigments formed could be made because of the amounts of bile pigment obviously retained in the tissues From the amounts found in the blood and urine it would appear that about three times more pigment I was formed than bilirubin The total amount of bile pigments formed after complete removal of the liver was not greatly different from that found at comparable times after biliary obstruction Hepatectomy in the rat also showed the accumulation of pigment I in the blood and somewhat smaller accumulation of bilirubin The urine was also found to contain pigment I

I also have determined the serum concentrations of pigment I pigment II and bilirubin in a number of persons who had hyperbilirubinemia of some degree Of the serums which contained some direct reacting bile pigment by the van den Bergh method that from persons with only two

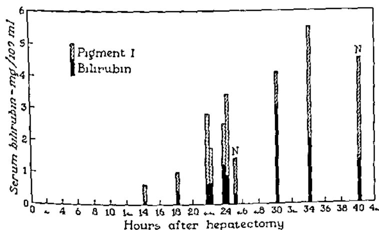


Figure 1 Bile pigments of serum of dogs determined the indicated number of hours after complete removal of the liver The height of each column represents the total bile pigments and each rectangle the amount of pigment I and bilirubin N denotes that bilateral nephrectomy was performed also at the time the liver was removed No pigment II was found in the blood and only pigment I was found in the urine after hepatectomy

exceptions has shown only a single pigment in the direct reacting portion Four persons considered to have constitutional hepatic dysfunction and one with icterus due to chronic passive congestion from cardiac failure were found to have only pigment I in addition to the increased bilirubin in their serum Serums from persons jaundiced from other causes that is from hepatitis cirrhosis cholelithiasis carcinoma of the pancreas carcinoma of the liver and chlorpromazine contained both pigment I and pigment II Since pigment I is formed outside the liver and pigment II is formed only by the liver the relative proportions of each retained in the blood might be expected to give an index of the efficiency of the liver in converting pigment I and bilirubin to pigment II Retention of pigment II would be considered as impairment of excretion of bile whereas retention of pigment I would be considered as failure of the liver to remove this pigment from the blood with or without failure of the excretory process Such information would be of value in the differential diagnosis and treatment of obstructive and hepatic types of jaundice

Billing reported determinations of the three bile pigments in the serums of 14 patients with obstructive jaundice In these pigment I constituted 38 to 6 per cent of the total pigment pigment II from 15 to 35 per cent and bilirubin from 18 to 39 per cent The mean for the group was pigment I 49 per cent pigment II 6 per cent and bilirubin 5 per cent Three patients with hepatitis were found to have more pigment I than pigment II or bilirubin in their serums Billing's figures afforded no separation of these two types of jaundice However she found that after ligation of the biliary duct the concentration of pigment II in the serum of rats was considerably elevated in relation to that of pigment I and bilirubin

My findings in 23 patients with hepatocellular disease were similar to those reported by Billing The concentration of all three bile pigments was elevated in the serum and pigment I was the predominant pigment present Twelve of these patients were in the acute phase of infectious hepatitis during the first 3 weeks of the disease and of the 11 who had cirrhosis 3 cases were definitely of the postnecrotic type In these patients the distribution of the three bile pigments of the serum appeared to bear no relation to the total bile pigment present

In serums from 3 of 27 patients jaundiced because of biliary obstruction I found that pigment II was the predominant bile pigment present whereas in four more of pigment I was present Fourteen of the 27 patients were suffering from obstruction due to carcinoma 6 from obstruction due to stones in the common duct and 7 from benign stricture in the common duct The distribution of the three bile pigments in the serums was not related to the total pigment present The relative amounts of pigment II and pigment I present in the serums of these patients were tabulated as the percentage of pigment II present in the sum of both direct reacting pigments I and II and that 100 minus the per cent of

pigment II is the per cent of pigment I The percentage of pigment II and the percentage of patients with each group of lesions are shown in Figure From values obtained for the per cent of pigment II in the serums of the 50 patients in these two groups a fair separation of groups is apparent The clinical diagnosis of each of these patients was made prior to the separation of bile pigment In retrospect the differential diagnosis between obstructive jaundice and hepatocellular disease would have been materially aided in several instances if serum in which pigment II constituted less than 50 per cent of the total pigment had been considered as indicative of hepatocellular disease and serum in which pigment II constituted more than 50 per cent of the total pigment had been considered as indicative of biliary obstruction

I have no valid explanation for the fact that my group of patients with biliary obstruction showed more pigment II than did the 14 patients reported by Billing I do not believe that differences in techniques are responsible for the discrepancy The technique closely followed that of Billing My findings in patients with hepatitis closely correspond to her findings in that disease as was the case also in 4 of our 27 patients with obstructive jaundice

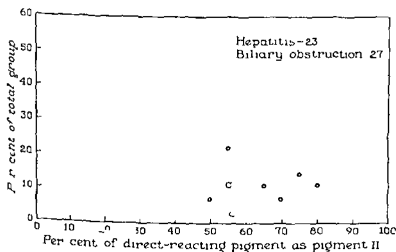


FIGURE 2 Distribution of direct reacting pigment II in the serum of patients suffering from liver disease On this chart the percentage of the total direct reacting pigments (I and II) in the serum that was pigment II is plotted The percentage which was pigment I may be estimated Each dot represents the per cent of the 23 patients having hepatocellular disease in whom the percentage of direct reacting pigments in the serum that was pigment II was near the indicated figure The open circles represent the percentage of the patients having biliary obstruction with the corresponding values of distribution of pigment II in the serum Three of the 4 patients represented by the 2 dots marked C were given [illegible] of [illegible] cirrhosis

SUMMARY

The recent demonstration of the presence of three bile pigments in most icteric serums necessitates a revaluation of the concepts of jaundice Pigments I and II both react directly with the van den Bergh reagent, are excreted in the bile and may be excreted in the urine Physiologic studies indicate that pigment I and bilirubin (indirect) are formed outside of the liver and that conversion of these two pigments to pigment II occurs only in the liver Following uncomplicated obstruction of the common bile duct of animals pigment II predominates in the blood which indicates the continued conversion of pigment I and bilirubin to pigment II With continued biliary obstruction larger amounts of pigment I appear in the blood The partition of the three bile pigments of icteric serums from patients suffering from hepatocellular disease or from obstructive jaundice was determined in 50 cases In this small series it appeared that retention of a large portion of pigment I was associated with hepatocellular disease and that larger amounts of pigment II were retained when biliary obstruction was present

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The Diagnosis of Hepatitis by Needle Biopsy

EDWARD A. GALL, M.D.

(Cincinnati, Ohio)

The contribution of the needle biopsy method to the understanding of hepatitis needs no elaboration. No longer in the forefront of clinical investigative procedures in this field, biopsy observations have nonetheless left an indelible imprint upon medical concepts. The older controversial hypotheses stemming from conjecture and from study of the fatal lesions at necropsy now rarely intrude themselves. The biopsy method has served to establish the identity of hepatitis and, perhaps of greater significance, has provided means for the appraisal of clinical phenomena and the assessment of laboratory results. A reflection of this is the heightened assurance and accuracy with which the diagnosis of viral hepatitis is now made.

Only a small number of jaundiced patients present serious problems in differential diagnosis. It is impossible to estimate the actual proportion of correctly recognized cases in which reservation existed in the mind of the clinician. However, if one evaluates only upon the basis of expressed opinion without concern for degrees of uncertainty, experience indicates an error of approximately 10 per cent in cases with suitable clinical data, competent examination and complete laboratory studies (Figure 1). It is the purpose of this paper to review the lesions appearing in biopsy specimens in this small group of patients in whom diagnostic error occurred with greater than occasional frequency. It is in these cases that the needle biopsy method has its major clinical application.

The histologic features of acute and chronic viral hepatitis have been authoritatively described earlier in this symposium. A brief tabular resume is necessary in order to provide a base line for contrast purposes (Table 1). It is noteworthy that despite variations in degree there are two constant features: a universal portal area exudate and accompanying evidence of parenchymal cell injury in one form or another (Figures 2, 3, 4, 14, 15). In the variant of hepatitis characterized by a prolonged course occasionally simulating obstructive jaundice[1-3] the reactive portal area exudate assumes a prominent position in the histologic process

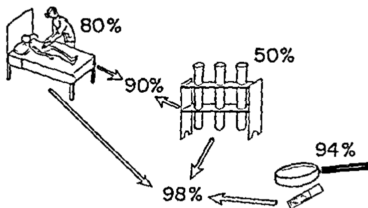


FIGURE 1 Percentages indicate accuracy of diagnosis as determined by history and physical examination, laboratory tests and needle biopsy examination alone and in combination.

TABLE 1

VIRAL HEPATITIS — HISTOLOGIC FEATURES

Parenchyma	*Portal Area*
Balloon cells	Universal exudate
Pseudoacini	Lymphocytosis
Mitoses	Mild eosinophilia
Hyalin cell	Undisturbed ductule
Focal necrosis	Pseudoductal formation
Lymphocyte satellitosis	Pericholangitis
No fat	Kupffer cell pigment
Bile stasis	

the parenchymal alterations, though persistent, may be difficult to demonstrate (Figure 8). To this pattern, in which inflammatory elements appear more heavily concentrated in the area occupied by the interlobular ductules, the term "pericholangitis" has been applied.[1] This is a purely descriptive expression and should not be taken to indicate a specific lesion (Figure 4). It certainly does not regularly accompany any special train of clinical events.

Many patients with infectious mononucleosis exhibit jaundice and most of them have laboratory evidences of liver impairment.[4, 6, 8, 14, 3, 31]

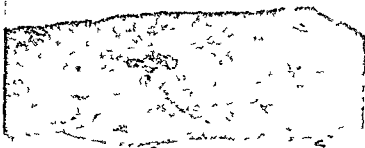


Figure 2 Acute viral hepatitis. Low power view of a needle sample showing characteristic universal prominence of portal areas due to inflammatory infiltration. Also of note is the absence of fatty vacuolization.



Figure 3 Acute viral hepatitis. Parenchymal elements are swollen, multinucleated and contain pale staining cytoplasm. Cell columns are thickened and irregular. A few scattered lymphocytes may be seen.

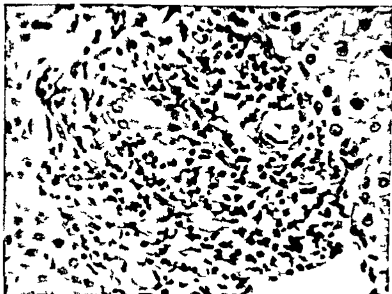


FIGURE 4 Prolonged viral (cholangiolitic) hepatitis Lymphocytes tightly gathered about an intact interlobular ductule comprise the lesion designated pericholangitis

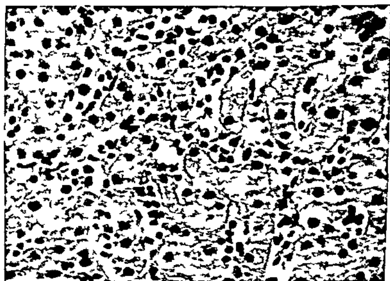
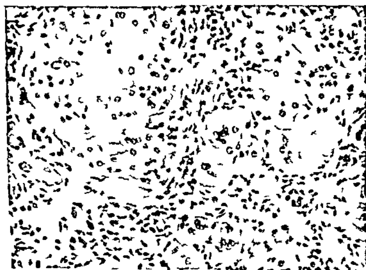


FIGURE 5 Infectious mononucleosi Large numbers of atyp cal lymphocytes thro the sinusoids into unusu l prom nence Li er cells here are essentially unremarkable in appearance else here they exhib t changes not unlike tho e observed in mild viral hepatitis

The hematologic manifestations however usually suffice to clarify the diagnosis On the other hand the presence of a disturbed peripheral blood picture in hepatitis has caused some confusion in differentiation The cases of mononucleosis in which histologic studies have been carried out have shown hepatic alterations almost identical with those seen in viral hepatitis[13, 24] There is not uniform agreement that the portal area exudate is specifically characterized by the presence of atypical lymphocytes In this location the cells do not lend themselves to clear cut cytologic definition A much more characteristic finding is the presence of variable numbers of lymphocytes here recognizably abnormal in many of the hepatic sinusoids (Figure 5) This actually constitutes the sole means of distinction in florid cases

Occasionally individuals are encountered with seeming acute viral hepatitis apparently in an initial attack who upon biopsy are found to have well advanced cirrhosis of one or another variety (Figure 6 and Figure 7) There is evidence of recent activation with manifestations of both cellular damage and inflammatory exudate and in a few frank parenchymal necrosis has supervened These represent instances of latent cirrhosis obviously antedating the appearance of the jaundice which presumably



[illegible] Hepatic necrosis with nodular regeneration (subacute hepatitis) Small groups of swollen multinucleated parenchymal cells reflect [illegible] regeneration These are embedded within thin stroma the seat [illegible] inflammation Patient with jaundice of two-week duration was [illegible] to have acute hepatitis

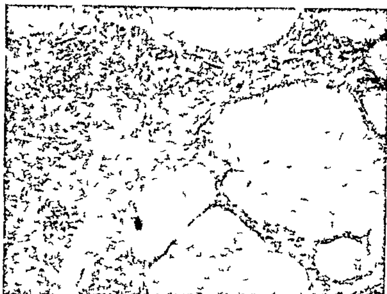


FIGURE 7 Postnecrotic cirrhosis with active chronic hepatitis Advanced nodular regeneration with broad intervening scars The latter contain large numbers of reactive inflammatory cells Presence of cirrhosis unsuspected patient believed to have acute hepatitis

represents either a complication of the underlying process or an exacerbation of it The episode attracting attention may so closely resemble acute viral hepatitis that if biopsy had not been made a suspicion of cirrhosis would never have existed Many cases cited in the literature as examples of cirrhosis following hepatitis may actually represent instances of this nature [1, 18, 1, 4] Although taken to support the belief that cirrhosis constitutes one of the sequelae of hepatitis a concept which may well be a valid one it is obvious that documentation with direct biopsy during the apparent initial phase of the disease is essential

A decreasing number of patients with extrahepatic biliary tract obstruction require biopsy clarification There are a few however who exhibit equivocal manifestations and in these the clinical diagnosis and proper therapy await pathologic study The tissue alterations of obstructive jaundice are well known [1, 3, 30] Unfortunately many reports emphasize the features of the severe and fatal forms and in such situations the clinical diagnosis is rarely unclear In those patients with early obstruction in whom the complications of ductal infection have not yet developed the morphologic problem may be somewhat more difficult This is particularly the case in attempting a distinction from the borderline instances of prolonged hepatitis Under these circumstances certain

negative observations may assume significant importance Notable is the absence of parenchymal cell alteration of the type seen in viral hepatitis (Figure 8 and Figure 9) The polygonal cells have a quiescent appearance although a small number may contain intracytoplasmic fat vacuoles Centrilobular bile precipitation with capillaries may be no more striking than is seen in hepatitis but is invariably present (Figure 9 and Figure 10) Foci of feathery degeneration (Figure 11) and bile lake formation (Figure 1) findings pathognomonic of obstructive disease are not encountered in the mild or early phases One may note on the other hand minute foci of necrosis about intercellular bile plugs with loosely gathered reactive neutrophiles (Figure 13) These are in sharp contrast to the satellite clusters of lymphocytes surrounding shrunken eosinophilic cytoplasmic masses observed in hepatitis (Figure 14 and Figure 15) Portal areas contain a relatively small inflammatory exudate which varies in intensity from lobule to lobule and may be entirely lacking in some lobules Concentration about interlobular ductules suggests the pericholangitis of hepatitis but neutrophiles predominate and characteristically migrate into the wall of the ductule (Figure 16) Intraductular suppuration (Figure 17) microcalculus formation (Figure 18) and bile duct proliferation are not usually observed at this stage When ductular proliferation does occur it may be distinguished from pseudoductular budding



FIGURE 8 Prolonged (cholangiolitic) hepatitis Note the pseudoacinar appearance of parenchymal cells about central vein Inflammatory reaction and bile stasis is scant Intracellular fat absent

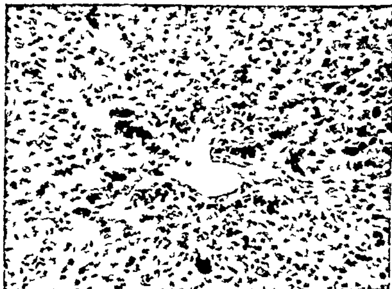


Figure 9 Obstructive jaundice common duct stone Intercellular plugs of inspissated bile fill the bile capillaries surrounding the central vein Although there is some cellular disintegration in this region the parenchymal elements remain small and relatively unaffected



Figure 10 Prolonged (cholangiolitic) hepatitis Here too capillary bile stasis is a prominent feature Note however that parenchymal elements are disturbed and there is a considerable inflammatory (lymphocytic) exudate



FIGURE 11 Ol tructive jaundice carcinoma of pancreas Feathery degenera t n is characterize l by attenuation and disappearance of liver cell v th a reticular residuum arranged radially about a central zone in which frank necrosis has occurred

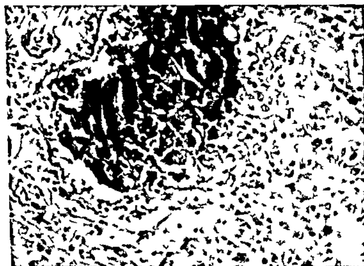


FIGURE 12 Ol tructi e jaundice common duct stone A more ad anced les n th n that ol served in Figure 11 l iberated secreti n n forms a deeply pig mented b le lake in the center of the zone of feathery degenerat n



FIGURE 13 Obstructive jaundice carcinoma of papilla of Vater A small coagulum of liberated bile and necrotic cells is surrounded by a neutrophile exudate Uninvolved cells lack the alterations common in viral hepatitis

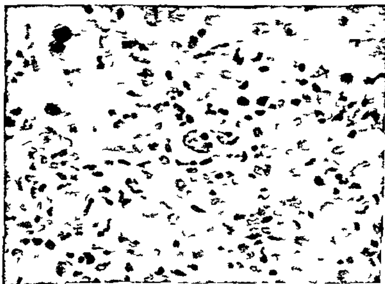


FIGURE 14 Viral hepatitis A shrunken cell (Mallory body) in process of necrosis Separated from adjacent elements the cytoplasm is homogeneous and eosinophilic the nuclei pycnotic Reactive lymphocytes are loosely gathered about the cell

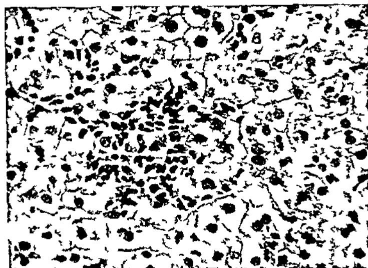


FIGURE 15 Viral hepat tis Focal individual c ll necros s The cell exciting the reaction is ob cu red by the satellite cluster of lymphocytes and h stiocytes Neutrophiles are conspicuou ly absent



F URE 16 Obstructiv jaun lice c mmon d ict t ne Acute cholangitis is characteri e l by i iv t n f neutr philes int tl all c f the interlol ular du tul Neutrop hil s appear in the p r du tal reg on as vell



FIGURE 17 Obstructive jaundice with acute cholangitis common duct stone Portal area bile duct proliferation is accompanied by severe edema and neutrophile exudate Inflammatory cells are also observed in the walls and lumens of the affected ductules.



FIGURE 18 Obstructive jaundice with microcalculus formation carcinoma of pancreas Biliary ductular proliferation with dilatation and precipitation of static bile



FIGURE 19 Prolonged (cholangiolitic) hepatitis Portal area is enlarged inflamed mildly fibrotic and exhibits pseudoductular proliferation Note the intact and quiescent interlobular ductule to the left

of hepatitis since in the latter the interlobular ductule itself remains intact (Figure 19) and in the former it does not This ordinarily cannot be evaluated in a single portal area but requires a survey of several

Chlorpromazine methyl testosterone and certain other agents with increasing therapeutic application have resulted in the appearance of jaundice in a small number of patients suffering from a wide variety of nonhepatic ailments[3 5 7 ... 37] Although presumably related to drug administration in certain of these cases suspicion of biliary obstruction or coincidental hepatitis may be difficult to allay Unfortunately the biopsy may not be overly helpful Centrilobular capillary bile precipitates with only minor evidence of parenchymal disturbance comprise the essence of the lesion (Figure 20) However careful search will usually reveal evidence of focal liver cell degeneration with minute clusters of lymphocytes and in a few cases spotty portal area round cell infiltration not unlike pericholangitis (Figure 20 and Figure 21) Although there are no indications of septic exudate and both the ductular system and most of the parenchymal cells lack a restless appearance it is obviously difficult to distinguish this lesion from those of mild obstructive disease or subsiding hepatitis Knowledge of the special clinical and therapeutic circumstances in a given case is essential to proper interpretation of this condition by the pathologist

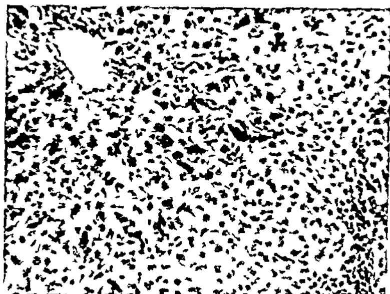


FIGURE 20 Methyl testosterone jaundice The lesion is highlighted by plugs of inspissated bile festooned about the central vein Parenchymal cells are un remarkable A mild infiltration with lymphocytes is present in a nearby portal area



FIGURE 21 Thorazine jaundice In this specimen too there is centrilobular bile plug formation not shown in this illustration The portal area here is the seat of lymphocytic aggregation about the interlobular ductule (pericholangitis) The parenchyma appears quiescent

In municipal institutions where alcoholism constitutes the accompaniment of many unrelated diseases the icteric alcoholic often poses a serious problem in differential diagnosis This special group consists of individuals who present themselves with deepening jaundice having recently enjoyed a prolonged drinking bout When a clear history is obtained suspicion of what we have chosen to term toxic hepatitis is readily aroused Such a history however is not always available Laboratory tests often provide an equivocal spectrum of results and alcoholics are far from immune to other forms of hepatic disease

The biopsy specimen is most helpful here for the lesion is very different from that encountered in other icterogenic conditions The liver is exceedingly fatty but the character of the lipid appears to differ from that observed in the nutritionally deficient individual without jaundice or other manifestations of hepatic deficiency The large lipid vacuoles often have a foamy microvesicular structure (Figure 22) The inner aspects of the vacuoles are not sharply defined but are smudgy and ragged In formalin fixed tissues artefact pigment is characteristically deposited in the fuzzy area serving to highlight this feature The fat is irregularly distributed in the lobules but may involve all parenchymal elements It is common to find variations in cytoplasmic staining and

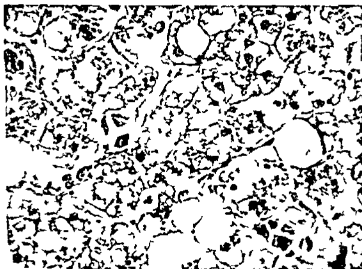


Figure 22 Acute toxic hepatitis Liver cells are swollen and obviously fatty Many cells exhibit microvacuolation and intense granular pigment deposit Margins of the vacuoles are fuzzy and often marked by the presence of formalin pigment

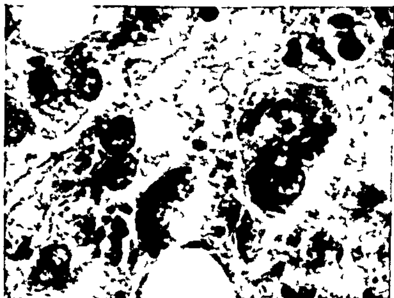


FIGURE 23 Acute toxic hepatitis alcoholic intoxication Swollen liver cell contains smudgy eosinophilic introcytoplasmic material (alcoholic hyalin)

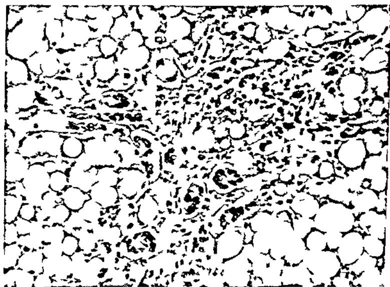


FIGURE 24 Toxic hepatitis early cirrhosis A stellate portal area contains an essentially normal centrally placed interlobular ductule but is fringed by cellular cords resulting from pseudoductular proliferation The latter probably reflects response to injury in the surrounding fatty parenchyma

so called alcoholic hyalin characterized by an ill defined but refractile eosinophilic intracytoplasmic deposit is often evident (Figure 3) Here too there is capillary bile plug formation and Kupffer cells are thrown into prominence by a heavy content of lipid and pigment Focal necrosis is a common feature in the more severe forms of this lesion This is accompanied by a contiguous neutrophile exudate Portal areas are swollen edematous and may exhibit stellate configuration Inflammatory cells are frequent but are not present in excessive numbers Neutrophiles usually predominate and are intermixed with round cells Although the interlobular ductule is unaffected certain of these cases reveal pronounced pseudoductular proliferation probably reflecting response to parenchymal cell necrosis (Figure 24)

Serial study has shown that toxic hepatitis of this type is a potentially reversible lesion in patients who have recovered only minor residual stigmata may be found Others however have shown progression with extensive necrosis and have succumbed in hepatic coma There is good reason to believe that this process may indeed represent one early phase in the pathogenesis of nutritional cirrhosis The number of patients with toxic hepatitis who proceed in this fashion cannot be estimated It should be noted that the lesion is not limited to individuals who are alcoholic it may appear following exposure to certain other hepatotoxic agents Indeed even in the patient with evidence indicating alcohol poisoning the lesions may not represent the direct effect of alcohol at all Persons so addicted are notorious for the bizarre nature of substitutes with which they may experiment

The conditions described constitute the bulk of those which have offered some diagnostic difficulties Table and Table 3 contain the

TABLE 2

DIFFERENTIAL DIAGNOSIS OF JAUNDICE

	Viral Hepatitis	*Obstructive Jaundice*	*Toxic Hepatitis*
Lobular Parenchyma			
Balloon cells	+ +	0	±
Pseudoacini	+	0	0
Hyalin cell	+	0	0
Hyalin necr	0	0	+
Focal necrosis	+	+	+
Lymphocyte satellitosis	+	0	0
Neutrophile satellitosis	0	+	+
Fat	0	±	+ +
Bile stasis	+	+ +	+
Bile lake	0	+	0

Table 3

DIFFERENTIAL DIAGNOSIS OF JAUNDICE

	Viral Hepatitis	*Obstructive Jaundice*	*Toxic Hepatitis*
Portal Area			
Universal exudate	+++	±	+
Lymphocytes	+++	+	+
Eosinophiles	±	0	±
Neutrophiles	+	++	+
Fibrosis	0	±	±
Ductule			
Disturbed	0	± – ++	0
Proliferation	0	± – ++	0
Pseudoductule	±	0	+
Exudate	0	± – ++	0
Pericholangitis	++	±	±

cytologic characteristics which aid in differential diagnosis There have been a small number of other diseases which though rare deserve some attention

Pylephlebitis resulting from an obscure septic process in the portal system may provide momentary diagnostic difficulty In our biopsy studies there have been three instances of jaundice with hepatocellular features one due to Bacteroides infection and two to infection with the coliform group of organisms Microscopic inspection of the small portal area venules demonstrates the pathognomonic lesion of septic venous thrombosis accompanied by perivascular neutrophilic exudate (Figure 5) Parenchymal elements may be entirely unaffected or may exhibit pyemic abscess formation

Jaundice is of course a common occurrence during the newborn period Few such patients are susceptible to the transcutaneous needle biopsy method but a number have biopsy material procured during exploration for supposed biliary obstruction Among the conditions recognized by this means has been the debated disorder giant cell hepatitis [9 10 1 30 3] The cytologic features of this condition characterized by enormous multinucleated parenchymal cells require no extended discussion (Figure 6) It should be noted that this allegedly unique cytologic feature is not limited to children but has been encountered with varying intensity in adults as well The range of cellular atypism in adults with this phenomenon extends from multinucleated balloon cells commonplace in epidemic hepatitis to giant parenchymal elements identical with those seen in the newborn cases (Figure 7) Some of the adult patients have been considered to have classical viral hepatitis

During recent years increasing attention has been given to certain inborn errors of metabolism which may be manifested by icterus [11 19 33]

FIGURE 25 Pylephlebitis bacteroides septicemia An interlobular portal venule is occluded by a septic thrombus

FIGURE 26 Giant cell hepatitis of the newborn Well shown are the enormous, misshapen multinucleated parenchymal cells. Cytoplasm contains a heavy dusting of granular pigment In this area bile plug formation is not shown In the upper right corner may be seen a cluster of nucleated red cells.

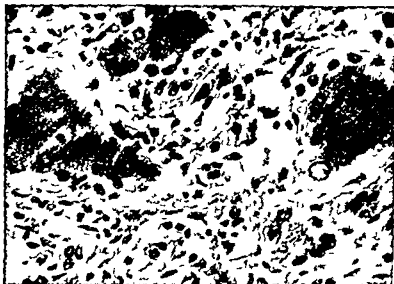


FIGURE 27 Giant cell hepatitis in an adult Cells are identical with those shown in Figure 26 Patient was a 45 year old man believed to have hepatitis induced massive hepatic necrosis

Although galactosemia has been recognized for almost 50 years its importance as a cause of serious even fatal hepatic disease has only had general recognition for the past decade The disease is one which can be efficiently handled therapeutically and prompt recognition may be life saving The lesions produced presumably as the result of the toxic effects of improperly metabolized galactose are not specific Liver cells exhibit evidence of profound injury with severe fatty change extensive necrosis and even advanced cirrhosis (Figure 28) A peculiar acinus like arrangement of injured parenchymal cells about droplets of inspissated bile (Figure 29) has been said to be characteristic of this condition but obviously is not The need for recognition of the disorder is evident That it may represent one of several hepatic derangements attributable to genetic abnormalities justifies further thought [10]

SUMMARY

Three conditions (viral hepatitis toxic hepatitis and biliary obstruction) comprise the more common affections offering difficulty in the differential diagnosis of jaundice Obviously there are many other diseases which also require consideration but their over all incidence is low and they are more readily distinguished by clinical manifestations In all of these however the needle biopsy method provides a relatively safe and effective means of recognition



FIGURE 28 Galactosemia with hepatic necrosis A 6 week old infant with non fermenting reducing sugar in urine and profound jaundice Vacuolated parenchymal cells form small clusters lying within a fine intralobular type of fibrosis

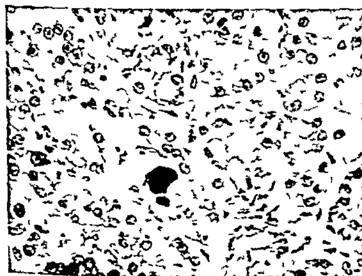


FIGURE 29 Galactosemia with hepatic necrosis A plug of inspissated bile is surrounded by abnormal hepatic cells

Clinical appreciation of viral hepatitis particularly when proper history and physical examination are supplemented by judiciously selected laboratory procedures has attained a high level of accuracy In no small measure this reflects the influence of biopsy studies carried out during the past 15 years Indeed at the present time only a small percentage of icteric patients require etiologic clarification by biopsy

The criteria for the histologic differentiation of viral hepatitis from those conditions which commonly resemble it are shown in Table and Table 3 Distinction of infectious mononucleosis cirrhosis with jaundice pylephlebitis metastatic neoplasm and many other parenchymal processes *is also readily made by this means On the other hand any inference that* the histologic method is free of pitfalls is certainly unjustified A difficulty exists in assessing the peculiar lesions encountered in patients who develop jaundice following the administration of methyl testosterone and chlorpromazine Microscopic interpretation here must have the adjuvant benefit of clinical data

This however is not an unmixed blessing since at least one source of pathologist error is the undue influence of misleading clinical implication Contributory also are the experience and critical capacity of the microscopist The ability to avoid interpretation of specimens unsuitable for analysis by reason of size fixation or technical inferiority is in direct relation to these features

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DESIGNATED DISCUSSION

HECTOR DUCCI, M.D. (Santiago, Chile): The usual procedure for evaluating the help of the laboratory in the differential diagnosis of jaundice is to compare its results with the final diagnosis. Sometimes the admission laboratory report is used; in other investigations the peak value of the laboratory tests during the course of the disease is utilized.

In our last 200 cases of hepatitis (according to the diagnosis at the moment of discharge) we have compared the admission bedside diagnosis with the first report of the commonly used laboratory tests (prompt direct and total serum bilirubin, total cholesterol, alkaline phosphatases, cephalin cholesterol flocculation test, thymol turbidity and flocculation and colloidal red reaction).

The bedside diagnosis was made, written and signed by the physician actually in charge of the patient. The purpose of not using a special diagnostic team was to have a true over all picture in a large medical service. The attending physician did not know the laboratory report until he had sent his written diagnosis to us.

The results of the comparison between the admission bedside diagnosis of hepatic jaundice and the first laboratory report are shown in Figure 1.

It can easily be observed that in 34 instances the attending physician did not make the admission diagnosis of hepatic jaundice. In 17 cases he diagnosed posthepatic jaundice and in 18 he did not commit himself

CONTRIBUTION OF THE LABORATORY TO THE DIAGNOSIS OF HEPATIC JAUNDICE IN 200 CASES WITH FINAL DIAGNOSIS OF HEPATITIS

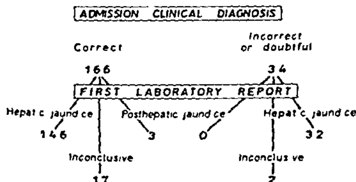


FIGURE 1

In 32 of those 34 cases the first laboratory report was for hepatic jaundice and in it was inconclusive Of the 166 cases with a correct admission bedside diagnosis of hepatic jaundice the first laboratory report was for hepatic jaundice in 146 for posthepatic jaundice in 3 and inconclusive in 17

In summary in none of these 200 cases of hepatitis were both the clinician and the laboratory simultaneously wrong All the cases in which there was a discrepancy between both methods were subjected to additional studies and the correct diagnosis was made None of these cases was subjected to surgical exploration

It is interesting to note that a complete agreement between all the tests used and the diagnosis of hepatic jaundice was obtained in only 97 of the 200 cases (48 5 per cent) Nevertheless the laboratory was interpreted as indicative of hepatic jaundice in 81 more cases slight discrepancies between the different tests were disregarded and the laboratory diagnosis of hepatic jaundice made according to the over all laboratory picture This gives a total of 178 cases (89 per cent) with a correct admission laboratory diagnosis versus a total of 166 cases (83 per cent) with a correct admission bedside diagnosis

If only one laboratory test is considered the agreement between its first report and the final diagnosis in these 200 cases varied between 61 per cent for alkaline phosphatases and 9 per cent for the cephalin cholesterol flocculation In spite of the apparent value of this last single test the isolated performance of one flocculation reaction is not to be recommended in the differential diagnosis of jaundice Unpredictable changes in sensitivity can easily be depicted by the simultaneous use of other tests Moreover the cephalin cholesterol flocculation test gave a large number of positive results in a series of posthepatic jaundice cases studied during the same period in which the 200 cases of hepatitis here analyzed were observed

The number of cases of hepatitis in which the admission diagnosis of hepatic jaundice was not made may seem too high Nevertheless the age distribution of our cases could account for it In the whole group of 200 cases only 95 or 47 5 per cent were below 30 years of age and in the group of 34 cases with an incorrect or doubtful admission bedside diagnosis only 11 or 32 9 per cent were younger than 30 (Table 1)

As has been shown above the laboratory tests in common use in the study of jaundice help in the differentiation between hepatic and posthepatic types Nevertheless in cases of hepatic jaundice they do not give additional information regarding the acute or chronic character This differentiation may be of diagnostic and prognostic significance

There are some newer tests which help in the differentiation between acute and chronic types of liver disease with jaundice Among these

TABLE I

AGE DISTRIBUTION OF HEPATITIS CASES

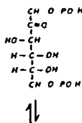
Years of Age	Total Group (200)	Cases with Incorrect or Doubtful Admission Bedside Diagnosis (34)
10–20	10	1
20–30	85	10
30–40	55	5
40–50	22	6
50–60	15	7
60–70	7	4
Over 70	6	1

aldolase phosphohexose isomerase transaminase and serum iron are the most widely used at present

Dr Wroblewski has already reported his work on transaminase and we have previously published our results employing the serum iron determination The enzymatic action of aldolase is shown in Figure 2

ENZYMATIC ACTION OF ALDOLASE

FRUCTOSE 16 DIPHOSPHATE



DIHYDROXYACETONE PHOSPHATE GLYCERALDEHYDE PHOSPHATE

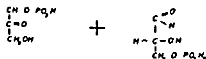


FIGURE 2

We have determined the aldolase activity of serum according to the method of Sibley and Lehninger in 156 patients

Acute hepatitis	60
Cirrhosis of the liver	44
Posthepatic jaundice	52

In 47 of the cases of hepatitis serum iron was also measured by the method of Barkan and Walker

In apparently normal subjects studied by us the values of serum aldolase had a mean of 4 08 mm³ H D P per milliliter (units) with a range of 1 75–11. In the 60 cases of hepatitis considering only the admission laboratory examination the values of serum aldolase had a mean of 27 65 units with a range of 3–90

If we consider values of over o units of serum aldolase as of diagnostic significance 68 3 per cent of our cases of hepatitis and 3 8 per cent of our cases of posthepatic jaundice had figures of this magnitude None of the apparently normal subjects or of the cases of cirrhosis had values of over o units

In Figure 3 the combined results of serum iron and serum aldolase determinations in cases with different types of hepatobiliary disease with jaundice are illustrated Only the cases of hepatitis show a combination of high aldolase and serum iron values The association of aldolase over 20 units and of serum iron over oo gammas is seen only in cases of hepatitis

The course of aldolase values in relation to those of bilirubin and iron

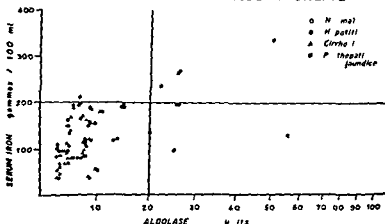
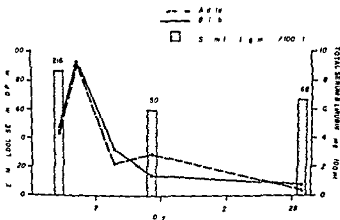


FIGURE 3

in a typical case of acute hepatitis is shown in Figure 4. Aldolase and *bilirubin follow a parallel course reaching normal values almost simultaneously*; on the contrary, serum iron remains elevated for a longer period.



Fi lrf 4

Panel Discussion on Differential Diagnosis

HENRY BOCKUS M D *Moderator*

SHEILA SHERLOCK M D (London England), CHARLES S DAVIDSON M D (Boston Massachusetts) CHARLES JOHNSTON M D (Detroit Michigan) VICTOR M SBOROV M D (Redwood City California) JOHN R NEEFE M D (St Petersburg Florida) LEON SCHIFF M D (Cincinnati Ohio) HECTOR DUCCI M D (Santiago Chile) G A MARTINI M D (Hamburg Germany) and FELIX WROBLEWSKI M D (New York New York)

CHAIRMAN BOCKUS The first question is *When should one suspect hepatitis in the preicteric stage largely on a clinical basis?*

DR SBOROV I asked Dr Bockus to give me all the questions that stood on end This is a stiff one and apparently it is standing on end The diagnosis of viral hepatitis in the preicteric stage is of course an extremely difficult one to make because the preicteric stage of hepatitis can mimic the prodromal symptoms of many infectious diseases

The patient may manifest only fever malaise and anorexia which are very common and nonspecific complaints With the coming of jaundice or preceding the jaundice dark urine may be noted and one then becomes much more suspicious of the diagnosis of hepatitis I think that dark urine is perhaps the key finding or the key complaint of the patient in the prodromal stage of hepatitis

The dark urine may occur from two to four days prior to the time clinical jaundice appears and really focuses attention on the liver

Frequently an enlarged and tender liver is found at this stage and thus the localization of the disease in the liver can be more certain At this time too one would want to look to the laboratory for diagnostic liver function tests and as has been shown by Dr Neefe and Dr Reinhold the turbidity and flocculation tests are often positive in this stage If one is fortunate enough to have the serum glutamic oxaloacetic transaminase test available it might be used as an indication of liver dysfunction

CHAIRMAN BOCKUS Which of these newer tests that you have been hearing so much about this morning does the panel feel has the greatest promise in the diagnosis of the preicteric stage of viral hepatitis?

DR DUCCI I think any of the enzyme tests but I would recommend the colorimetric method for transaminase because it is simpler

Dr Sherlock I think the three most important points in making an early diagnosis of virus hepatitis are finding both a tender liver and bilirubin in the urine by a sensitive method such as the tablet test or Fouchet's method, in the presence of a normal sedimentation rate

Chairman Bockus Did we have a clear understanding from Dr Wroblewski as to just how soon the transaminase may be anticipated as being positive in viral hepatitis in the human What is the timing on that Dr Wroblewski

Dr. Wroblewski The longest time in an epidemic situation where the diagnosis would be suspected that we have observed is about 6 days In other words, 6 days before the patient develops symptoms there were changes in the enzyme test which suggested that the patient was going to develop hepatitis

This is in contradistinction to the concept of the incubation period of this disease It would appear that some of these patients have a longer incubation period, either by virtue of the size of the inoculum, or by virtue of the immunity but in any case we have observed it anywhere from 65 days to shorter periods prior to the development of symptoms.

Chairman Bockus Is it the feeling of this panel that this test is going to be utilized by all of you from this moment on, in any patient who has been in contact with this disease Is that your feeling Dr Neefe

Dr Neefe I would think that we undoubtedly still need to document this test It sounds very promising but it does not seem to me that there is yet sufficient evidence to know just where it fits into the picture

Chairman Bockus Are there any other comments about this facet of transaminase

Dr. Sborov While we are discussing this with Dr Wroblewski, I would like to ask if he has had any experience with the use of this test in intrahepatic cholestasis, such as occurs in chlorpromazine jaundice or viral hepatitis manifesting this feature and whether the transaminase is elevated in this disease Presumably there is very little liver cell necrosis in these conditions, and one would wonder whether the transaminase would be helpful.

Dr. Wroblewski We try to use reassurance rather than thorazine In those patients whom we have observed with thorazine intoxication, the serial changes and quantitative changes in the serum enzyme are not un

like those that are depicted in toxic hepatitis This of course does not fit the generalization that thorazine is primarily an intrahepatic obstructive agent In any case we have observed serial and quantitative changes in thorazine toxicity which reflect changes associated with toxic hepatitis

Chairman Bockus We have a question from Dr Brick to Dr Davidson The term hepatitis superimposed on cirrhosis was used by one of the essayists Does the panel recognize this entity? If so how is it recognized? Does viral hepatitis occur superimposed on cirrhosis or fatty liver

Dr Davidson Dr Brick it is very nice to know that you are here but as you know perfectly well I can t answer this question Maybe some of the other members of the panel can but as you have addressed it to me I will try Almost every patient who comes to the Boston City Hospital with cirrhosis and jaundice suggests to the intern a diagnosis of cirrhosis of the liver with superimposed viral hepatitis

I looked on this for many years as meaning that the intern didn t know that this is an unlikely combination of events Viral hepatitis is an unusual disease in our community compared with cirrhosis so this combination seemed to me to be unlikely at least in the frequency with which the diagnosis was made

However looking at it a little more seriously it seems as Dr Gyorgy has pointed out that there may be more than one damaging event which leads to active cirrhosis that is cirrhosis with jaundice not only malnutrition and possibly alcohol itself but perhaps in some instances the virus of hepatitis may add its damaging influence

I am not pathologist enough to always be able to distinguish whether viral hepatitis exists in the liver ravaged by malnutrition and alcohol I find Dr Mallory who works with us is frequently unsure in determining in a patient who has severe active cirrhosis with fatty infiltration parenchymal disorganization necrosis fat and so on whether a lesion suggestive of viral hepatitis is also present or not Hepatitis superimposed on cirrhosis may very well exist and I think it is a promising field for investigation when we can determine whether a patient is infected with the virus of hepatitis or not The fascinating studies of Dr Neefe concerning patients with cirrhosis and harboring at the same time the hepatitis virus are to this point

Chairman Bockus Dr Schiff I am sure is very anxious to step in at this moment

Dr Schiff I can recall one or two cases that we have seen at the Cin

cinnati General Hospital where Dr Gall has made the combined diagnosis on the basis of needle specimens of the liver I don't recall the type of cirrhosis at this moment Perhaps he might know We have seen it once or twice

CHAIRMAN BOCKUS Would anyone else care to participate in this question?

DR NEFFF I would like only to recall that such a combination conceivably is quite possible as illustrated by one of the blood donors that Dr Norris Dr Reinhold Dr Murray and I had a chance to see This donor was an alcoholic and was picked up simply because his blood had caused jaundice in a recipient His blood was proved by Dr Murray to contain hepatitis virus and at the same time on biopsy he had the changes of what would be called a cirrhosis by most pathologists

I think it takes a very expert pathologist to interpret needle liver biopsy and to *distinguish* between the finer degrees of advanced active disease regardless of the pathology Certainly in this case I would not know whether it was the alcohol or the virus or both that was responsible It may be that several things are needed to produce this combination

CHAIRMAN BOCKUS Dr Martini does it happen in Germany?

DR MARTINI I have never seen it but I believe Dr Popper mentioned it in one of the Macy conferences I don't know whether he would comment He made a statement that it is apparently very rare in his material.

DR SHERLOCK I have seen this on one occasion only This was a 32 year old man who had infectious hepatitis in an epidemic in the army and was shown by biopsy afterwards to have developed cirrhosis Three years later he had a sufficiently severe gastric ulcer to merit a gastrectomy At the gastrectomy a biopsy of the liver was taken which showed an inactive cirrhosis He was transfused at the same time Three months later he developed what seemed to be a typical virus hepatitis biopsy did show additional features with cellular infiltrations and necrosis, which were interpreted as acute virus hepatitis imposed on cirrhosis This association must be exceedingly rare

CHAIRMAN BOCKUS How long does this virus live in the liver once it gets there Dr Sherlock?

DR SHERLOCK I wish I knew

CHAIRMAN BOCKUS Doesn't that enter a little into the discussion, Dr Neefe?

DR NEEFE I don't think there are too much data available but Dr Stokes had a carrier, I believe, who had evidence of carrying the virus for 3 or 4 years at least and that is based on the fact that his blood caused jaundice in recipients and some years later was shown to be still infective for volunteers

So presumably it can be carried around, whether in the liver or not I don't know, but somewhere over long periods

CHAIRMAN BOCKUS I think this next question is really deserving of rather general comment Do any of the panelists ever diagnose and manage a case of jaundice on purely clinical observations

Isn't that a nice question? Must you always have these many laboratory determinations? Is the bedside facet here entirely a thing of the past?

DR SBOROV In my experience the answer is No! I don't think it is safe to manage, if you will, a case of jaundice purely from a clinical point of view — at least I do not usually feel that secure with the diagnosis There is too much overlapping in the differential diagnosis, particularly when one is considering the differences between medical and surgical jaundice or the hepatic versus the posthepatic type of jaundice There is too much to be lost, I think, by waiting in a case of obstructive jaundice when one is wrong in his diagnosis Or, vice versa, there is a great deal to be lost, even the life of the patient, if one diagnoses a case wrongly as an obstructive jaundice and operates when one is dealing with viral hepatitis

I would say from general experience in all branches of medicine that one cannot practice too good medicine and that one should make as full use as is necessary of all the laboratory and histologic data that are available in order to establish a diagnosis and then use whatever laboratory means are needed to manage the patient

CHAIRMAN BOCKUS Dr Schiff, suppose you were shipped off to some out of the way place to practice medicine where you didn't have any laboratories and you saw people with jaundice What do you suppose your batting average would be in the differential diagnosis of viral hepatitis versus posthepatic obstructive jaundice?

Let's say you observe the patient for three weeks You wouldn't operate under three weeks anyway in all likelihood unless there was some very crucial thing present Considering your ability and experience in liver disease would your batting average be very low?

DR SCHIFF I can tell you what my batting average was but not on a deserted island free of laboratory facilities

In a series of 84 patients this series comprising patients with jaundice of various types I saw the patient within 24 hours of admission before any laboratory tests were done There was no opportunity for cheating I made the diagnosis in writing This was in a general hospital It proved to be correct in 84 per cent of the cases

I compared my own accuracy with that of the resident staff I was a little higher than they were I think they were in the high 70s In a few instances where I was wrong they were right and vice versa

I think if a clinician takes a careful history and does a careful physical examination stressing particularly certain features in the examination of a jaundiced patient he should arrive at a diagnostic accuracy probably of around 80 per cent

CHAIRMAN BOCKUS Dr Schiff in that same group of patients when you went on and added your diagnostic procedures were you 100 per cent right? Didn t you have a few patients explored and make a mistake?

DR. SCHIFF I think in that particular group we reached a diagnostic accuracy with the aid of laboratory tests and needle biopsy specimens and so on of a little over 93 per cent

CHAIRMAN BOCKUS I think they do a little better than that in Chile don t they Dr Ducci?

DR DUCCI I think with prolonged observation any case of jaundice can be diagnosed The patient either dies or recovers Confining ourselves to the hepatitis problem I think the age group is most important In patients under 20 years of age you can make the diagnosis and treatment over the telephone You don t even have to see the patient In a large medical service however I think the help derived from a good laboratory —and I have to say good twice — is of tremendous help

CHAIRMAN BOCKUS Dr Davidson did you have a comment to make?

DR DAVIDSON I wondered if Dr Ducci s telephone system transmitted color I would agree with him that age is very important In jaundice of the older age group of 70 80 and 90 years the history is often not very good nor very accurate the patient doesn t remember well Physical examination should help us and often does but patients of that age group often have more than one illness which serves to upset the diagnosis con

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of saving hospitalization and I do not think that ever ought to enter into this picture

Actually I have obtained very little help in general evaluation of a patient with obstructive jaundice from the laboratory findings It must be recognized that one has to have a whole battery of laboratory findings and these must all be put together and added to the viewpoint that one gets from examining the patient and from a good clear cut history

As far as the referral of the patient is concerned the surgeon of course is in the situation of seeing the patient with viral hepatitis only after he has been gone over many times and the patient with obstructive jaundice may not be seen until many months have elapsed So by the time the surgeon sees it it is usually a pretty clear cut picture

I have been concerned with the problem of thorazine jaundice We have had several of these people who have been jaundiced for a long period of time and without at any time the prostration that goes along with the usual viral hepatitis So the signs of hepatitis are pretty well masked At any rate I think that all too much is made of the fear of operating on patients with viral hepatitis if one is not sure of the diagnosis

We forget sometimes that the needling and all the other diagnostic procedures and the suspense carrying on over a long period of time sometimes are much more devastating than a simple operative procedure Many times operation is a simpler procedure than some of the things which are done to try to find out whether or not to operate

In those instances in which I have been mistaken on thorazine jaundice the patient has not suffered because of the operative procedure We have decided pretty definitely at the time what the problem was Usually this can be done with the history and the physical examination and it is rare that one does have to operate on the patient

What I am about to say I say with some trepidation because a surgeon isn t supposed to be dealing with virus hepatitis However several of our surgeons here in Detroit myself among them have had hepatitis and we being interested somewhat in the general field of liver diseases have assayed without saying anything to our medical friends to determine the bile salt content of the blood In the acute phase of hepatitis one does find (and this is in the acute phase only) that there is retention of bile salt in the blood which so far we have not found in any other instance even in instances of cirrhosis of the liver

If in addition to determining the bile salt content of blood one gives a little stimulus by the administration of ox bile the content of bile salt goes remarkably high because it is not excreted as rapidly in hepatitis as it is normally

I don t know whether I have answered the question without raising

siderably I find in that age group accurate diagnosis is difficult and I am sure my diagnostic percentage is well below that of Dr Schiff

Over the years of being interested in these patients I believe I am less and less able to be sure of differentiating medical and surgical jaundice than I was or thought I was

CHAIRMAN BOCKUS Dr Schiff relents a little bit and thinks he should have mentioned to you a couple of items in history and physical examination which were partially the intent of the question

DR SCHIFF I really wanted to stress a point or two in the physical examination In patients with viral hepatitis the liver usually extends below the right costal margin two to three finger breadths Sometimes it is not palpable and it is usually tender If a patient is seen whose liver is apparently *quite large and extends four to five finger breadths below the* right costal margin I think it is fairly safe to exclude viral hepatitis as a cause of that enlargement It does not hold all the time but it does hold in a great majority of cases

On the other hand we have found particularly in suspected neoplastic obstruction or invasion of the common bile duct by carcinoma of the head of the pancreas that if the patient is seen after he has been jaundiced for several weeks and his liver cannot be felt on careful palpation with proper relaxation that tends very strongly to exclude obstructive jaundice due to carcinoma of the head of the pancreas As was shown by Watson obstruction is complete in this type of jaundice in over 90 per cent of the cases so that bile stasis occurs the liver becomes engorged with bile and therefore should be palpable

CHAIRMAN BOCKUS Dr Johnston we haven t heard from you I suppose in Detroit you have very few patients referred to you for exploration *because the medical men are unable to make this differential diagnosis* between viral hepatitis and posthepatic obstructive jaundice

DR JOHNSTON In relation to the diagnostic aspects of this matter and the dependence on the laboratory I think one must remember that laboratory aids are nothing more than indications We should not be dependent upon them I doubt if anyone sitting in the laboratory without seeing the patient could ever make a clear cut diagnosis or differentiation between viral hepatitis and obstructive jaundice

Someone here mentioned one of the crucial points about evaluation of a patient i e the decision to operate upon the patient with jaundice does not need to be made hurriedly We don t perform emergency laparotomies for jaundice The only pressing problem would be the matter

it means nothing When you have this associated with a high blood sugar it is further suggestive at times

Assume we have done the G I series I think you said the patient was deeply jaundiced is that right Well whether justified or not I have always been a little fearful of doing a biopsy in a deeply jaundiced patient The few instances of bile peritonitis of which I am aware have usually been in such patients Perhaps some of the others on the panel have had the courage to do it under those circumstances I probably would not in the deeply jaundiced patient

Then we come to the next situation We still don t know and I think here I would either wait another week and see if something broke or give the patient a therapeutic trial of the adrenal steroids It is emphasized that such a therapeutic trial would be considered diagnostic only if this patient started to improve promptly and clearance of the jaundice was progressive and uninterrupted When such improvement does not occur or is only partial we still don t have a diagnosis and the final result would be an exploration

CHAIRMAN BOCKUS You would have the steroid therapy first The panelists should take for granted that we have done all the roundabout studies

I am glad you mentioned them Dr Neefe because they are important but each of us can t go into them in detail We will presume that all the general diagnostic studies for ruling out carcinoma and so on have been done Biliary drainage too if you wish so we can get to the meat of the thing What is done in Germany under these circumstances?

DR MARTINI I would go more into the history before the onset of jaundice developed I think some symptoms should be mentioned that is arthralgia which occurs in about 20 per cent of our patients with viral hepatitis Then I would ask for rashes and if it happened to be a person who smokes I would ask him whether he still likes his cigarette because that is rather a sensitive sign If he has not stopped smoking it is very much in favor of extrahepatic obstructive jaundice

In the blood we are looking for virocytes That helped us many times In viral hepatitis there are types of mononuclear leukocytes which are very similar to those that one can find in mononucleosis We do a leukocyte concentrate as it is called and make a smear of the leukocyte concentrates and very often find virocytes

I would also comment on the iron The iron diagnostic test is useful for a differentiation between hepatocellular and obstructive jaundice But it is not so helpful in differential diagnosis of intrahepatic or extrahepatic obstructive jaundice

Recently we have done sodium outputs in acute hepatitis and we

doubt about the integrity of our internists They do a pretty good job I think because we see very little hepatitis and most of the time when they refer the patient it is because the jaundice is obstructive in nature

CHAIRMAN BOCKUS I am going to try to summarize about six questions There is a lot of interest in a clinical way on the solution to this problem and I am going to make it very specific to see if we can really get some decisions made

The hypothetical patient has been jaundiced for three weeks The jaundice is deep Many clinicians think a decision concerning the need for surgery ought to be made at about three weeks The usual flocculation tests the serum alkaline phosphatase and cholesterol point toward an obstructive type of jaundice either within the liver (medical) or outside the liver (surgical)

What additional tests if any can be used at that time to come to a decision Should Dr Watson s fecal urobilinogen test be utilized? Does the differential level between the concentration of the serum bilirubin and the serum phosphatase have value in this instance Does the addition of the galactose transaminase serum iron and aldolase have value here? Which one should we do first and when?

Let us canvass the panel again and decide what each one of you is going to do Let us decide which additional tests have value and what your preferences may be If decisions cannot be made with certainty after all tests do you employ liver biopsy under these circumstances? If that doesn t settle it will you explore?

I am going to start with Dr Neefe and if each of the panelists takes three minutes to tell us precisely what he does in this situation it should prove most instructive

DR NEEFE I would like to make it a little more complicated and assume that we have had all the tests that have been mentioned this morning and we still don t know — which happens frequently I am sure

CHAIRMAN BOCKUS That brings us up to biopsy operation or nothing

DR NEEFE The next thing I would do if I hadn t done it already would be a gastrointestinal (G I) series because sometimes that is diagnostic and would have been had it been done several weeks earlier

I have seen patients without pain with pancreatic lesions who have a high amylase or lipase even though they have had no symptoms suggestive of pancreatitis I think when you find that in this picture it is very strongly suggestive granted that it often will be negative and then

very reliable when present On a number of occasions when the patient failed to relax his abdomen and we were not sure whether or not we were palpating the gallbladder we called in an anesthetist who administered sodium pentothal By this means we have had good relaxation of the abdomen and we have been able to satisfy ourselves as to whether or not the gallbladder was distended This has helped us very much in our decision

I think probably the most reliable index of obstructive jaundice is a combination of negative flocculation tests and a steadily rising serum alkaline phosphatase and serum bilirubin

In the past week I was called in to see a patient at the end of six weeks of jaundice who was being treated for hepatitis Her liver biopsy specimen was interpreted as cholangiolitic hepatitis Because abdominal pain was prominent and the flocculation tests were negative in the presence of a concomitantly rising serum bilirubin and serum alkaline phosphatase surgery was advised in spite of the biopsy findings The patient turned out to have a tumor of the pancreas

There is one point about needle biopsy in cases of jaundice If you know the patient has obstructive jaundice there is no point in doing a needle biopsy It won t tell you the cause of the obstruction Needle biopsy has its greatest danger in obstructive jaundice because of the chance of bile peritonitis occurring as a complication

If I am satisfied at the end of three weeks that everything points one way I might wait another week repeat the alkaline phosphatase the serum bilirubin and the flocculation tests If it still looks like obstructive jaundice I would advise surgery

If there is a discrepancy in the laboratory findings some pointing one way and others pointing another I think one is justified in doing a needle biopsy of the liver I will tell the patient in advance Look! We may be able to decide whether you need an operation or not by this procedure If we get into trouble an operation may have to follow With that attitude we have proceeded

As a matter of fact in the early days of liver biopsy before we were aware of bile peritonitis as a complication of obstructive jaundice we obtained needle specimens in thirty consecutive cases of obstructive jaundice without mishap

CHAIRMAN BOCKUS Will someone on this panel discuss the value of the oral galactose test in this differentiation Suppose you do an oral galactose test in doubtful cases and you get back 1 0 per cent urinary excretion Would that sway you in one direction or another When did you have a galactose test done on a patient of yours the last time Dr Schiff?

DR SCHIFF Not for a number of years

found that in patients with intrahepatic obstructive jaundice with all liver function tests negative the sodium output is disturbed so that may help in some cases in differential diagnosis The sodium output in hepatitis is rather low and it goes up once the bilirubin comes down

I would not have the patient operated upon before eight weeks

CHAIRMAN BOCKUS You would wait eight weeks?

DR MARTINI Yes There is nothing to be lost unless he is itching

DR DUCCI Answering specifically Dr Bockus s question and supposing the history has been well taken and the routine tests have been made in this case I would perform a serum iron and of course serial studies of urobilinogen Urobilinogen in the feces is most helpful In a great majority of the cases of hepatocanalicular jaundice simulating obstruction there is always some urobilinogen present in the feces On the contrary there is none in the cases of complete obstruction due to carcinoma

At this stage of the game if the serum iron is high and if we find some urobilinogen in the feces I would wait but if the serum iron is low and the urobilinogen is absent I would perform a surgical exploration

I don t mention the enzymes at the moment because in most cases by the third week they are already low or only very slightly elevated even if the case is one of hepatitis

We think that biopsy is contraindicated in a case of deep jaundice in the third week We also think that steroid therapy for diagnostic purposes is contraindicated

CHAIRMAN BOCKUS I am going to ask you just one question because I know of your experience with the serum bilirubin and the phosphatase level Do you pay much attention to that Suppose for instance this patient at the end of three weeks had a serum bilirubin level of 35 mg per cent and the phosphatase was 1 Would that influence you as compared with a serum bilirubin of 16 and a phosphatase of 35 Do you pay any attention at all to this disproportionate increase in bilirubin and phosphatase in this differential diagnosis?

DR DUCCI We pay some attention to it but it does not hold as well in children as Dr Shay has shown

DR SCHIFF I am assuming that all the tests are in agreement and are favoring obstructive jaundice I am assuming that a careful examination of the abdomen has not revealed the presence of a distended nontender gallbladder This sign pointed out by Courvoisier many years ago is

and tal e the history again I would do this perhaps several times and I would do a complete physical examination all over again This simple diagnostic means has helped me on more than one occasion

Having done that several times and still being in doubt I would wait if there was any thought that hepatocellular disease was likely just as Dr Sherlock has said

Dr Watson I believe introduced a very simple test very roughly qualitative for stool urobilinogen using Schlesinger s reagent and Ehrlich s reagent In our hands at least this has helped the differential diagnosis a number of times

The simple matter of measuring urine urobilinogen has sometimes been of help The finding of a high urine urobilinogen a dark cherry red color after Ehrlich s reagent is added is indicative of hepatitis when obstruction is high grade

Dr Sborov To get down to the real meat of your question which is this Do you do a liver biopsy first or do you explore first

One of the primary indications for liver biopsy is to differentiate medical from surgical jaundice It has been my practice to do the liver biopsy I hesitate and am apprehensive but I do them if other contraindications are not there namely bleeding I think others have done the same thing

We have encountered bile peritonitis following liver biopsy in non jaundiced patients as well as in the jaundiced ones It is a calculated risk and it i one that I personally would rather take than to have the patient undergo the morbidity of a surgical exploration

Unfortunately the liver biopsy is not the final answer In many of the hepatitis cases the liver biopsy looks exactly like extrahepatic obstructive jaundice but there are patients who pre ent the clinical picture that has been discussed here — that of a complete biochemical pattern of obstructive jaundice in which the histological findings are those that Dr Smetana call the standard picture of viral hepatitis It is in these cases that one uses the biopsy for diagnosis

If one finds on biopsy the standard type of hepatitis with a diffuse infiltrate through the liver one can avoid surgical morbidity and even mortality

So I think I would have liver biopsy high on my list if the other contraindications for biopsy were not there However even then one might find the histologic picture of obstructive jaundice and one would turn the patient over to the surgeon for the final answer

Chairman Pockls I am sorry to have to ask you another question Will you give me the timing on this All of these things have been done including the liver biopsy It is now three weeks since the onset of th

Dr Sherlock More note must be made of the mode of onset If particular attention is paid to this point you can nearly always make a correct diagnosis The intrahepatic obstructions whether they be due to drugs or to virus hepatitis have a relatively acute onset The patient feels ill is jaundiced within a matter of hours whereas in the extrahepatic group the jaundice comes on gradually and the patient does not feel so ill

The next point is that even without measuring fecal stercobilinogen if we just took the trouble to inspect the stools every day the answer would often be forthcoming

However if at the end of three weeks there is still diagnostic doubt I think aspiration liver biopsy is indicated Most of the group that have the obstructive features with a virus hepatitis basis as Dr Gall showed do have minimal parenchymal changes in the liver Frank virus hepatitis is diagnosed quite easily

I would certainly wait if there is any doubt at all The odds are that if you are in doubt the diagnosis is going to be hepatocellular jaundice An extrahepatic biliary obstruction is relatively easy to diagnose as brought out by Dr Ducci s slide Nearly all the cases in which he had any doubt were suffering from hepatocellular jaundice If doubt continues I would wait up to eight weeks before surgical intervention The dangers that follow operating on a hepatitis patient are too great to take the risk I have records of at least four patients with hepatitis who have died following exploration

Chairman Bockus Could you tell us briefly when those four died in relation to the onset of their disease — if you remember?

Dr Sherlock Within a week of the operation

Chairman Bockus Roughly how long were they sick Were they sick for two or three or eight weeks?

Dr Sherlock Some of them were operated on too soon at two weeks, but some of the others went up to about six weeks Equal numbers either way

Chairman Bockus Dr Davidson you haven t said anything for a long time

Dr Davidson I have very little left to say At the risk of being presumptuous I would say that the first thing I would do as others have said more or less would be to go to the patient s bedside close the door so as to shut out noise and extraneous factors and sit and spend an hour perhaps

is no reason to hurry I think we should always remember that One should be sure however before the problem is settled that if necessary the patient should be operated on to find out what he has

I believe an exploratory laparotomy should be done when people have something that looks like a serious disease and when the surgeon doesn t know what it is He should find out because I think it is incumbent upon us surgeons to find out and if it is necessary to do surgery to do it

We also forget that sometimes (and I think this is very pertinent) patients can no longer endure continued medical therapy We always forget that aspect of it I remember a specific instance of an individual who had obstructive jaundice and who also had diabetes I saw him after he had been on the wards for a month He was making no progress but was losing ground instead and I said he should be explo ed

A month later I was asked to see him again at a tumor clinic because it was suspected then that he had an obstruction to his bile outflow He was out of management as far as his diabetes was concerned The internist said that unfortunately the man had gotten to a state where he could not stand surgery

They called upon me and I said It s unfortunate but I am afraid this man will stand very little more medical therapy and I think we had better operate on him tomorrow which we did and we found a pancreatic cyst He is well and fine now

The point is that one fits the operation to the patient and does not simply do a widespread exploration and evisceration of the patient So I think one has to evaluate how the patient is doing If the patient is losing ground on medical therapy something else had better be tried though there is a strange notion that if a patient dies on the medical ward he is not quite as dead as if he had died on the surgical ward

Now I suspect that in patients who die a week or so postoperatively from hepatitis — if the statistics were gone over it would probably be found that more than 4 or 5 of them would have died without any operation and yet they wouldn t have excited any concern at all because they hadn t been operated on I think it is strange that we have this rather odd concept

That brings me to another point I personally don t like needle biopsy and the reason is that I don t feel I can control it as well

DR SCHIFF Do you think the mortality from an exploratory laparotomy is less than that of a needle biopsy?

DR JOHNSTON I am sure I can control things better if I see the liver and see what it looks like It can be done under a local with a small incision and you can take out a piece of liver and sew up the liver

jaundice Are you going to have the operation now in four weeks six weeks or eight weeks Do you consider it dangerous to procrastinate too long in a patient with obstructive jaundice? Do they occasionally get cholangitis so that the risk is actually increased after a certain period of time and if so what is that time?

DR SBOROV I see no particular danger in procrastinating a week or two if one is so inclined but I see no reason to do so If the liver biopsy shows the picture that is consistent with obstructive jaundice I see no reason to wait longer and I would recommend surgery without further delay

CHAIRMAN BOCKUS Dr Johnston your question is going to be a little bit different I would like you to comment on what you have heard if you wish but I would like you to tell us whether there are any special ways of preparing a patient of this type whom you are exploring who might conceivably have viral hepatitis Do you go through any special maneuvers in an attempt to get the patient in the best possible condition? Do you use antibiotics Do you prepare them with steroids? Is there any thing special that you do on your service when you are about to operate on a patient who might possibly have hepatocellular jaundice rather than posthepatic obstructive jaundice?

DR JOHNSTON In relation to the question which you asked Dr Bockus I might say that the surgeon wants to get the patient in as good a nutritive state as possible With obstructive jaundice that is not quite as difficult as with a patient who has been severely harassed by viral hepatitis over a long period of time because strangely enough the usual patient with obstructive jaundice still has some ability to eat and enjoy his food even though he has no bile in his intestinal tract — and one can correct for that of course

Aside from that we prefer to get the patient in as good general shape as possible correcting things that need correction such as anemia and if there is no need for correcting anemia there is no need for a blood transfusion or whatnot

Quite obviously we want to be sure he is not going to bleed and also that his vitamin K is up Outside of that I see no great need of fuss and stew about preparation that would not be done already on the medical service before he come to us

There is one point I would like to make here and that is that again usually in the case of obstructive jaundice whether it is from malignancy or not there is no great hurry to operate upon these patients So if there is something someone needs to do there is no big rush about it Or if there is any big concern about the patient's general well being there still

Dr. Ducci: Once would be enough.

Chairman Bockus: It isn't enough, is it, if you have a patient who has viral hepatitis? Don't some of them go through a phase of complete obstruction?

Dr. Ducci: I suppose the patient has been studied for the three weeks that preceded the decision.

Chairman Bockus: You just presume that the whole thing was obstructive in its concept?

Dr. Ducci: Yes — that the urobilinogen has been absent. As a matter of fact, if we compare the results of biopsy with the serum iron test for differential diagnosis at this point, serum iron is much better. There is nothing more difficult than to make a diagnosis between posthepatic jaundice and hepatocanalicular jaundice, the one that does not have any cell damage. They are diagnosed by chemical means. "Ever" and "never" are very dangerous words in medicine.

Sometimes we perform an operation. We haven't in the last 200 cases of hepatitis because we do more tests. We know more about the problem and we rely more on the results we have.

Chairman Bockus: I guess what you are saying is that you think now the combination of the iron and the aldolase is going to make things easier, particularly the iron?

Dr. Ducci: Yes.

Dr. Schiff: I don't believe there is any test or combination of tests that will take the place of a needle biopsy of the liver to tell you what is going on in the liver.

In the second place, I think a pathologist who is experienced in interpreting liver biopsy material like Dr. Gall, whom we are fortunate to have in Cincinnati, can in the majority of instances differentiate cholangiolitic hepatitis from extrahepatic obstructive jaundice. There are some earmarks which are infallible. For example, if you see a "bile lake" in a biopsy specimen, you know there is extrahepatic jaundice.

If it isn't a clear cut case, I would not hesitate to do a biopsy if no contraindications existed. In a very high percentage of cases we would have the answer.

Chairman Bockus: I am sorry you don't do the galactose test, Doctor.

Dr Schiff Suppose you did that to 1000 people what would your mortality be?

Dr Johnston I don't know what it would be I don't think the mortality would have anything to do with the operation

Dr Schiff You might lose one or two of them?

Dr Johnston I think so

Chairman Bockus I suspect there is a definite difference of opinion about whether or not a needle biopsy ought to be done before exploration in doubtful cases I don't know why we can't go over the panel again from that particular standpoint to see what the final vote is Dr Neefe should a needle biopsy ever be done in this differential diagnosis in your opinion?

Dr Neefe I think one of the reasons for differences of opinion here has been that we keep changing the case a little bit all the time

Chairman Bockus It is a deeply jaundiced patient whose prothrombin would permit of liver biopsy It is now three weeks and all the tests leave us in doubt Are you going to be one of those to use the biopsy, or are you going to explore

Dr Neefe Again in this very deeply jaundiced patient with complete obstruction if it were obstructive jaundice I would favor looking inside

Dr Martini I would do a peritoneoscopy with a needle biopsy instead of an exploratory operation

Dr Ducci Before considering an operation there are some other tests to be done

Chairman Bockus I thought the question was that we had done all the tests and we couldn't make up our minds

Dr Ducci I spoke about serum iron and urobilinogen in the feces I don't think the color of the feces can give you any exact information The difference between 5 mg or 10 mg or 0 mg is most important in the differential diagnosis and cannot be judged by looking at the feces

Chairman Bockus On that point Dr Ducci how many times are you going to do that?

PART VI

Clinical Management

Moderator CECIL WATSON M D (*Minneapolis Minnesota*)

DR SHERLOCK Whoever heard of a surgeon doing 1000 operations and doing only a little biopsy when he was inside the abdomen

One other point In the preoperative management of these cases if there is any possibility that you might be operating on someone with hepatocellular jaundice protein ought to be withdrawn from the diet on the day before the operation and two days postoperatively I would certainly do a biopsy before surgery if there were any diagnostic doubt about the type of jaundice

CHAIRMAN BOCKUS Dr Davidson and Dr Sborov you have not changed your minds Our time is almost up I don t know whether Dr Johnston ought to be allowed to rebut once more or not but I am afraid not

DR JOHNSTON You have taken the vote and I am still ahead

CHAIRMAN BOCKUS In summarizing this particular question — and that is *about all we are going to be able to do — I think you have sensed that* the difference of opinion really isn t as great as it sounds I think we all still recognize that with the tests as they now exist there will still be an occasional patient in whom this differential diagnosis is not being made

There will be an odd patient in which all of these tests will of necessity not give us the answer I think I agree with Dr Ducci that if one of these jaundiced patients had had a transaminase determination when the *jaundice first appeared and he had had the advantage of the serum iron test* and the aldolase test and if a galactose test had also been employed (prior to the beginning of the fourth week) a decision for or against operation would most likely be correct I would like to suggest that the panelists revert to the use of the galactose test and add it to the group of tests under discussion It has great value in this differential diagnosis if it is performed during the first three weeks of jaundice I think all of us might occasionally do a liver biopsy It is only a matter of how frequently we will do it In the patient who is very deeply jaundiced we are going to be a little cautious about doing it At the end of three or four weeks most of us are going to think seriously about doing an exploration if the findings are commensurate with extrahepatic obstructive jaundice

I agree with Dr Johnston that we are not at this time at least in the tests we have been using so far quite astute enough to be always right Occasionally I send a patient to the surgeon at the end of the third or fourth week of apparent obstructive jaundice to make the diagnosis for me because I am afraid not to operate I don t think that is going to happen nearly as often in the future

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Management of Hepatitis

RODERICK MURRAY MD

(Bethesda Maryland)

Since the use of steroids and the management of coma are being discussed elsewhere on this program this report will be confined largely to some points of management which were followed in the treatment of bout 130 cases of hepatitis of varying degrees of severity which developed during the course of studies directed towards the development of methods of sterilizing blood and blood derivatives with respect to the agent of serum hepatitis These studies were terminated approximately two years ago and since most of the basic results have already been reported elsewhere[1] details of the individual studies may be omitted here except for mentioning that the subjects were all male volunteers over the age of 21 with the majority being below 30 Some individuals over 30 were initially accepted as volunteers but for the greater part of these studies the average range was between 1 and 30

These cases resulted from the administration of the following infecting materials which were being studied for the presence of the agent(s) of hepatitis

(1) Infected pool plasma
(2) Infected pool plasma treated This represents materials which remained infective after various attempts at inactivation of the viru
(3) Carrier material
(4) Thrombin

These materials have been described in greater detail elsewhere

One of the most striking features about hepatitis as it occurred in this group was the great variability in severity ranging all the way from inapparent infections which were identified only because of the clo e scrutiny and the periodic routine performance of liver function tests to cases of coma with a fatal outcome in three instances It is extremely difficult to evaluate the effect of any form of treatment on the course of such a variable illness unless the results are pronounced or the study ha been set up in such a way as to demonstrate the statistical validity of any difference noted between comparable test and control groups Carefully controlled studies are essential if worthwhile deductions are to be made Even so there are other differences which have to be contended with in evalu

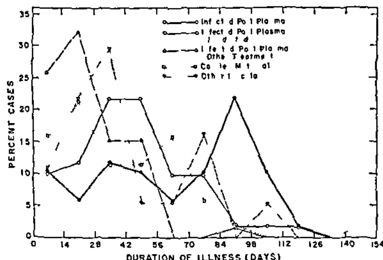


FIGURE 2 The severity of illness as sho n by durat on of illness in diffe ent groups f sul jects which recei ed different types of infecting material It ill be noted that the group hich received untreated infected plasma had a peak bet een 70 and days indicating a group of severely ill patients Peaks for the other infecting materials indicated shorter durations of illness

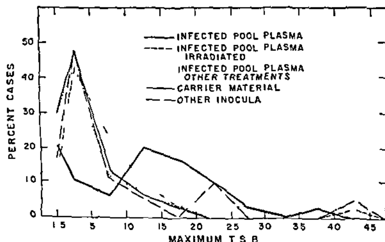


FIGURE 3 The distr bution of ma mum total erum bilirubin occurring during the illness of subjects tho received va ous nfect ng material The group which ceived untreated nfect ng pla ma sho ed a peak at approximatel 13 here the other groups h d a p ak b lo 5 ag in illustrating the variation in the se er ty of the illne e prod ced by d ff rent nfecting materials

ating the effect of therapy on the course of hepatitis For instance it is frequently stated that with infectious hepatitis the course of illness is generally shorter and milder than is the case with serum hepatitis The factor of age of course may be important here because infectious hepatitis is usually associated with lower age groups whereas homologous serum hepatitis tends to appear more frequently in older individuals The distinction does not stop here however as Figure 1 Figure and Figure 3 illustrate

There are differences in the character of illness caused by different infecting materials It is true that incubation periods may not be a significant characteristic here but duration of illness and the degree of icterus as measured by the total serum bilirubin level do appear to be involved

The essential features generally followed in the treatment of hepatitis are bed rest diet and the avoidance of additional trauma to the liver particularly by avoiding the use of hepatotoxic substances or drugs which might have an adverse effect on the liver In this respect of course drugs which are normally detoxified by the liver and which if not detoxified

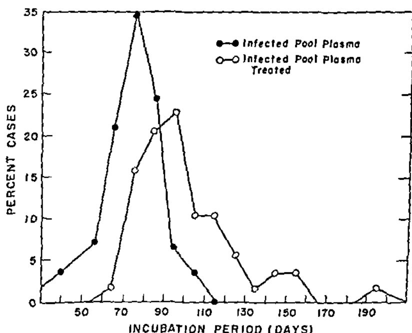


Figure 1 The distribution of incubation periods in two groups of subjects which respectively received untreated infected plasma and the same material which had been treated by various method designed to reduce infectivity It is apparent that there is a considerable lengthening of incubation period among the subjects that received the latter type of plasma

kept at bed rest with activity limited to visits to the bathroom until recovery was definitely under way at which time ad lib activity around the ward was permitted Any other course would have been irksome and difficult to administer

Diet The effect of diet on the course of hepatitis has recently received a considerable amount of attention [3] Chalmers *et al*[3] have shown that in infectious hepatitis at least a high protein diet resulted in a slight but *significant reduction in the average duration of the illness* There was no evidence from their studies that dietary supplements such as choline and vitamins contributed in this regard

The nature of the project upon which our group was engaged made it possible to design an additional controlled trial of the effect of the diet on the course of hepatitis Here a low fat high protein diet (oo Gm) was compared with a low fat low protein (103 Gm) diet in the treatment of 67 cases of homologous serum jaundice The numbers were small and the analysis was carried out with respect to both diet and the type of material which had caused the hepatitis in these cases This latter analysis was important and would not be possible normally except under exceptional circumstances when a single source infection was known Most of the comparisons concerning the effect of diet did not reach the level of statistical significance However one group of subjects gave evidence that at least the use of a high protein diet was associated with a prolongation of illness This comprised the largest number of cases developing illnesses after inoculation of the same material and it also represented the group with the largest number of severely ill individuals (See Infected Pool Plasma in Figure and Figure 3)

This result which appears at first examination in conflict with that obtained by Chalmers and his co workers with infectious hepatitis may be explainable by the severity of the illness in this particular group of patients and perhaps also by the high level of protein administered (200 Gm) or by the different characteristics of the illness This effect may not be unrelated to the type of experience reported by Davidson in the feeding of protein to patients with cirrhosis [4]

An interesting sidelight of this study was the analysis of the effect of the infecting materials which is illustrated in Table 1 and summarized from the above mentioned paper by Leone *et al*[2] The study indicates that the most important feature operating was a difference in the inocula received by the subjects a circumstance which points up what was mentioned earlier namely the variability of the illness both as to source and as to manifestations which makes it difficult to evaluate the role of any particular regime In view of the above findings it is recommended that the diet include about 3000 calories with approximately 150 Gm of protein

would accumulate on normal dosage schedules should be avoided or administered with extreme caution Treatment should be instituted as early as possible Since each case presents special problems no over all regime will be found fully satisfactory for all cases or for all stages of the illness Brief mention must be made of some of the therapeutic measures which have been used and examined in the treatment of hepatitis

Antibiotics These have not been demonstrated to have any significant value in the treatment of the average case although there seems to be some indication for using antibiotics such as aureomycin terramycin or neomycin in cases of coma or incipient coma In this series of cases aureomycin was used intravenously and where possible by mouth under such conditions but there is little evidence in examining these results or the results published in the literature that the use of antibiotics has any significant effect other than that of combating infection due to bacteria

Gamma globulin This appears to be without value in the treatment of hepatitis although it is very valuable in the prophylaxis of infectious hepatitis

Vitamin K This is frequently mentioned when the treatment of hepatitis is discussed It should be used in cases with any bleeding tendency associated with an elevated prothrombin time The nature of the illness suggests that this complication is to be expected in some cases at least In this series such a tendency was noted in only two cases

Activity Complete bed rest has long been an important feature in the management of hepatitis Patients who are seriously ill prefer to stay in bed and perhaps this is an instance of nature knows best Rigid bed rest has been prescribed in the past presumably on the basis that if a little of something is good more is better However the present treatment is to be more liberal as to the interpretation of bed rest and the rather detailed and well controlled studies made by Chalmers and his co workers[3] indicate that in the case of infectious hepatitis ad lib activity is to be preferred over rigidly enforced bed rest Early hospitalization is desirable and bed rest should be urged as long as acute symptoms persist Ad lib activity should be permitted once the symptoms have abated but patients should be restricted to the ward in order to avoid the possibility of undue exertion before they are ready for heavy exercise Patients may be discharged when the bilirubin has been below 1 5 and the bromsulphalein test at 45 minutes shows a retention of less than 5 per cent for a week or if the latter has become stabilized below 10 per cent

In this series of cases the effect of activity was not studied Patients were

ease In later cases if the subject had not become visibly jaundiced by the time the results of these tests were available a bromsulphalein test was performed and this gave excellent confirmation Zinc turbidity and thymol turbidity tests provided little useful information of value in either diagnosis or management Thirty per cent of this series of patients had consistently normal thymol turbidity level during the entire course of their illness The majority of the remainder showed only slight transitory elevations This may be a point worth considering in differentiating serum hepatitis from infectious hepatitis Our experience with the latter is limited but there has invariably been a brisk rise in those cases followed

Another factor in the course of a patient s illness which is awaited with some anxiety by the physician is the point at which improvement begins While the general well being of the patient often expressed by an improvement in appetite is helpful here in our experience the phenol turbidity test was most valuable in indicating improvement This was particularly so in severely ill individuals and a rise in this index preceded the fall in bilirubin by an average of some 3 days or more The cholinesterase test, which gave similar information was of interest to us particularly because of the stability of this enzyme We were able to retest serums which had been kept frozen for a year or more and obtain satisfactory results We have had no experience with the more recently reported enzyme tests of liver function but suspect that these will also have a similar usefulness

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TABLE 1

EFFECT OF INFECTING MATERIAL

Measure of Effect	Diet	Average			Remarks
		Infected Pool Plasma	Infected Pool Plasma Treated	Carrier	
		Days*			
Duration of illness (T.S.B.† 1.0 to 1.0)	High Protein	90	42	60	Significant effect
	Ad lib	67	56	39	Significant effect
Duration of illness (T.S.B. 1.0 to 1.5)	High Protein	72	24	45	Significant effect
	Ad lib	52	36	23	Significant effect
Duration from T.S.B. 1.0 to maximum	Both	24	15	17	No significant effect
Duration from maximum T.S.B. to 1.0	Both	53	34	33	Significant effect
Incubation period	Both	81	103	61	Significant effect
		mg./100 ml.			
Maximum T.S.B.	Both	15.4	8.0	10.1	Significant effect

* To nearest whole day
† Total serum bilirubin

and any amount of protein above this should be on an ad lib. basis. Intravenous glucose feedings are indicated during the period of anorexia in order to maintain the caloric intake and prevent dehydration. Fat in an amount of approximately 150 Gm. of egg, meat, or dairy product origin should also be given.

The value of liver function tests in detecting cases early in the course of illness was a matter of concern, and every attempt was made to hospitalize subjects as soon as a diagnosis of hepatitis could be made. Since these individuals had been inoculated with material which was infectious or potentially infectious, there was very little question about the actual diagnosis itself. The main problem was to make this as early as possible. These patients were kept under close vigilance and had almost immediate access to a physician when symptoms occurred. Regular periodic blood and urine examinations for evidence of liver dysfunction were carried out and even asymptomatic cases were recognized early. The group of tests used included 1 minute bilirubin, total serum bilirubin, zinc turbidity, phenol turbidity, thymol turbidity, thymol flocculation, cephalin flocculation, urine bilirubin and urine urobilinogen. We came to find the results of the 1 minute bilirubin test coupled with a positive cephalin flocculation test, which in a large percentage of cases became positive at about the same time, as the earliest reliable objective indication of the onset of dis-

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*Management of Coma**

CHARLES S DAVIDSON MD W H J SUMMERSKILL MD **
and STANLEY J WOLFE, MD †

(Boston Massachusetts)

As one might expect the pathogenesis clinical manifestations prognosis and presumable treatment differ considerably between the various diseases affecting the liver Thus the treatment of hepatitis of viral origin must differ considerably from the treatment of hepatic disease in alcoholics It is the latter if anything that we are qualified to discuss so that the material to follow is concerned chiefly with the cirrhosis of alcoholics and insofar as this is translatable to the hepatic coma seen in viral hepatitis and other liver conditions will depend upon elucidations of the mechanism of coma Protein withdrawal the treatment for hepatic coma emphasized in this paper is in our hands[1] as effective in cirrhosis of alcoholics as reported by Sherlock Summerskill and Dawson[2] for that of non alcoholics

It seems necessary to digress here to discuss briefly the clinical and pathological findings in cirrhosis of the alcoholic Two distinct clinical syndromes may be described although many patients fall somewhere between The most common of these is the classical patient with cirrhosis demonstrating severe undernutrition ascites abdominal collateral circulation and esophageal varices but who usually shows little jaundice and if there is any change in the leukocyte count leukopenia predominates These patients usually show massive hepatic fibrosis with nests of remaining or regenerating liver cells between which may appear quite healthy In contrast to this is the acute hepatic insufficiency of the alcoholic[3, 4, 5] Clinically these patients are usually jaundiced frequently have a polymorphonuclear leukocytosis and although portal hypertension with ascites and collateral circulation is commonly present it is not usually a pre

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** Rockefeller Traveling Fellow of the Medical Research Council of Great Britain.

† Public Health Service Research Fellow of the National Heart Institute

diet especially since the classic work of Patek and his colleagues[8] As well as vitamins emphasis in the diet has been placed upon the provision of ample high quality protein Although an adequate diet consumed by the patient is the best treatment of hepatic disease of the alcoholic paradoxically treatment of hepatic coma at present is complete protein withdrawal This cannot be considered optimum treatment of the basic liver disease but is only a temporary measure for the cerebral manifestations

The characteristic syndrome of impending hepatic coma mental confusion the flapping tremor and certain electroencephalographic changes can be induced in sensitive patients with liver disease by dietary protein and some other nitrogenous substances[9] Most evidence at present would incriminate ammonia as the most likely substance to arise from these nitrogenous materials either in the gastrointestinal tract or in some circumstances from the liver kidneys or other parts of the body and to be the toxic agent acting on the brain causing coma Treatment is thus devised to reduce the formation of ammonia and perhaps other toxic materials in the intestines and to allow or promote removal of ammonium in the body In addition to complete withdrawal of protein from the gastrointestinal tract broad spectrum antibiotics are administered orally and laxatives and enemas are used to empty the gastrointestinal tract This treatment has shown by far the best results in our experience Of 34 patients recently treated by this regimen 21 patients improved and 13 recovered sufficiently to be discharged from the hospital The best results were in the nonjaundiced chronic patients and the poorest when jaundice was deep Experience with glutamic acid cortisone and hydrocortisone administration to these patients — the former to combine with ammonium and the latter exhibited because of a reported excellent effect in some patients with viral hepatitis in coma — has given us little encouragement for their use alone or as an adjunct to protein withdrawal Furthermore lipoic acid which has been reported to reduce the blood pyruvate concentration so often elevated in hepatic coma has not given clear cut therapeutic benefit As intimated above the patients in whom gastrointestinal hemorrhage and other precipitating factors could be controlled and the patients with chronic cirrhosis have the best prognosis whereas the outcome is extremely doubtful for those alcoholics with acute hepatic insufficiency who have developed signs of early or deep hepatic coma

This regimen including the use of not only protein withdrawal but antibiotics and gastrointestinal tract emptying must be considered to some extent empirical At present it is impossible to decide the relative effectiveness of each of these aspects of the treatment although they are aimed at the same cause preventing absorption of toxic nitrogenous materials including ammonia from the gastrointestinal tract Protein withdrawal would seem to be the essential element of this treatment although to what

dominant finding In this situation although fibrous tissue may be and usually is present two other lesions of hepatic parenchyma are present in varying degrees The first of these is fatty change of the liver cells and the second parenchymal disorganization focal necrosis polymorphonuclear infiltration and the intracellular hyalin as described by Mallory [6]

As to treatment the chronic form responds slowly to the alcohol withdrawal and an adequate diet presumably by regeneration of liver cells The acute form may go on to a rapid improvement or a fatal termination likewise usually rapid The fatty infiltration presumably responds to choline methionine and other lipotropic agents consumed in an adequate diet whereas the etiology and treatment of the usually more severe cellular lesion are at present unknown

Hepatic coma may occur in any of these situations When it occurs in the chronic patients it usually arises from some circumstance such as high protein diet sedation gastrointestinal bleeding and the like which frequently can be controlled In this situation the coma may be reversed fairly easily and recovery can often be expected When a patient with acute hepatic insufficiency be it fat or other cellular change shows evidence of early hepatic coma the prognosis is grave and although temporary recovery to a normal state of consciousness may occur permanent improvement in this situation once coma has set in is uncommon

It is now generally believed that hepatic coma has something to do with deranged ammonium metabolism That this is frequently if not always true seems likely but the treatment of this condition is extremely complicated owing to the precipitating factors and a complex (or poorly understood) pathogenesis Hence treatment cannot be routine [7] Among the precipitating factors one must recognize the sensitivity of patients with liver disease to hypnotics analgesics sedatives and anesthetics to acute infection to gastrointestinal bleeding to operative procedures—even such minor ones as paracentesis—and to fluid and electrolyte disorders Treatment must begin with prevention keeping these and perhaps other precipitating factors in mind for patients with hepatic coma have extremely labile homeostatic mechanisms In addition to prevention and to exacting nursing care careful measurements must be taken of pulse blood pressure fluid intake and output as well as serum concentrations of nonprotein nitrogen and electrolytes Assuming that gastrointestinal bleeding and other precipitating factors are under control that fluid and electrolyte balance is corrected that the vital signs are not far out of line and that meticulous general care is being taken of the patient the problem chiefly revolves around reducing the formation of ammonia and perhaps other toxic nitrogenous substances from the intestines

Treatment of liver disease especially that of the alcoholic for some years has been based on the consumption by the patient of a nutritious

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extent the exhibition of antibiotics may be expected to prevent formation and absorption of ammonia and other nitrogenous materials is not known Faloon and his colleagues[10] in some careful studies have been able to feed otherwise toxic protein by the addition of oral neomycin in relatively large quantities The optimum antibiotic or course of antibiotic therapy has certainly not been decided and requires further investigation

A few more words might be said about intermediary metabolism in hepatic coma Bessman and Bradley[11] have pointed out the value of measuring arterial ammonium rather than peripheral venous in that much of the ammonia presented to the tissues is removed Nevertheless although arterial concentrations are more frequently elevated than those in the peripheral vein in hepatic coma the correlation is by no means as good as one would expect if this were the sole toxic agent Reduction in arterial values has usually occurred following protein withdrawal antibiotics and gastrointestinal tract emptying However in patients who went on to a fatal termination a subsequent and late elevation often occurred frequently associated with a negative arterial venous difference that is ammonium production from the body tissues[3] Moreover the uptake of ammonia by the peripheral tissues in patients with liver disease with and without hepatic coma was not as great as that in normal individuals and seemed most impaired during coma This suggests a defect in ammonia removal by the tissues Such a defect in intermediary metabolism is further supported by the observed rise in blood pyruvate and a ketoglutarate concentrations following the administration of ammonium chloride to patients with liver disease but not in its absence Several observers[1, 13, 14] have found these keto acids to be elevated in hepatic coma and it may be speculated from these data that such elevation may represent a defect in intermediary metabolism due to impaired removal of ammonia from the blood by a diseased liver and abnormal body tissues

Finally it should be emphasized that the regimen involving complete protein withdrawal and antibiotics is in our hands the most satisfactory treatment of hepatic coma It cannot be considered optimum as the best evidence is that in addition to alcohol withdrawal a nutritious diet including protein is the best treatment for liver disease of the alcoholic

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Treatment of Acute Hepatitis with Cortisone

HECTOR DUCCI M D RICARDO KATZ M D and HERNÁN ALESSANDRI M D

(Santiago Chile)

We have previously reported the complete recovery of several cases of fulminant hepatitis in coma following the use of high doses of cortisone[1] The following is an example of a recovered case of fulminant hepatitis

M G J #51/12983 This 16 year old male experienced his first symptoms consisting of nausea and malaise on September 15 1951 On September 1st rapidly increasing jaundice was noted and somnolence appeared On admission October 6th he was stuporous and deeply jaundiced hepatic dullness was very much reduced (3 cm in the midaxillary line) With the diagnosis of fulminant hepatitis treatment with cortisone was started On the following day the patient appeared in deep coma and the dosage of cortisone was increased On October 9th he regained consciousness and rapid improvement ensued

A summary of mental condition treatment given and laboratory results is shown in Figure 1

On October 16th 10 days after falling into coma and 7 days after recovering from it a liver biopsy was performed which showed preserved lobular architecture slight fatty infiltration and inflammatory reaction in the portal spaces (Figure 2) A second biopsy on February 7 1952 showed normal liver histology (Figure 3)

This patient is still under control showing normal clinical and laboratory findings

The results we obtained with the treatment of acute benign hepatitis with cortisone used for a short period of only 9 days have also been published[3] and we have also briefly reviewed the literature on the subject Since then some other papers on the treatment of benign hepatitis with steroids have appeared

Heilmeyer and associates also using a short period although longer than ours observed beneficial results Huber and Wiley[5] in their patients with acute hepatitis treated with cortisone observed a more rapid drop of bilirubinemia a definite improvement of appetite and a shorter

teenth day this cannot be attributed to water retention as the weight is maintained on the twenty first day On the contrary the control group shows an average weight loss of 2 Kg This difference is possibly due at least in part to the improvement of appetite in the treated cases

(2) The rapid and abrupt drop of bilirubinemia in the treated group as compared with the control one is shown in Figure 4 The basal arithmetic means are respectively 13 5 and 11 7 mg per 100 ml of serum On the fourth day the means are 6 02 for the treated and 9 08 for the controls and on the eighth day 3 59 and 5 63 respectively These differences are highly significant statistically $\frac{\chi}{\sigma} = 2\ 67$ (p = 0 75) for the fourth day and $\frac{\chi}{\sigma} =$ 86 (p = 0 45) for the eighth day This significance is also shown by the thymol test 656 (p = < 0 01) for the fourth day and 79 (p = < 0 01) for the eighth day

On the twelfth day the difference between the means is smaller (3 54 and 3 90 for the treated and control groups respectively) If two of the treated cases who experienced marked recrudescences of jaundice at the end of the cortisone treatment are excluded the mean bilirubin value of the treated group is 2 7 on the twelfth day (dotted line in Figure 4)

The reduction of the mean total bilirubin in percentage for each group is given in Table 1

The influence of cortisone on bilirubinemia is more apparent when the

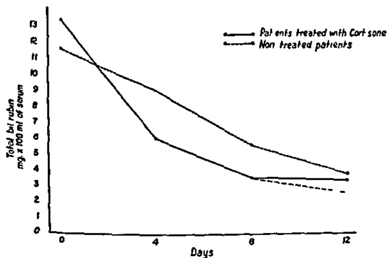


FIGURE 4 Course of ser n b l rubin n tr at d and contr l groups

Treated and control patients were kept in bed and received a diet low in fat (40 Gm) with no less than *1 Gm of protein per kilogram of body* weight and carbohydrates to complete a normal caloric intake No salt restriction or vitamin supplements were used Those patients with an odd admission number were treated with cortisone and those with an even one served as controls (*these did not receive placebo*)

Cortisone was given to the treated group by the oral route with the same scheme and the daily dose was administered in two halves every 12 hours first day 400 mg second and third days 00 mg fourth fifth sixth and seventh days 100 mg and eighth and ninth days 50 mg

Treated and control groups consisted of 41 patients each In the former the age varied between 17 and 49 years (mean 26 8), 3 were men and 18 women In the latter the age varied between 0 and 40 years (mean 8 1) were men and 19 women In 10 of the treated patients 1 Gm of chlortetracycline daily in four doses was added during the cortisone administration later this association was discontinued as it proved to be of no value

In the total group of 9 patients the following laboratory examinations were performed every fourth day prompt direct[9] and total[10] serum bilirubin cephalin cholesterol flocculation[11] colloidal red test[1] thymol turbidity and flocculation[13, 14] zinc turbidity[1] phenol turbidity[16] Takata Ara reaction[17] total cholesterol[18] and alkaline phosphatase[19] In the great majority of patients a study of the factors of the prothrombin complex feces and urine urobilinogen and serum electrophoresis were also carried out In 15 cases hepatic biopsy was done on admission but only in 8 (5 from the treated and 3 from the control groups) could a second one be performed from 8 to 22 days later Whenever possible patients were kept under observation until the total serum bilirubin was below 2 mg per 100 ml

Results

The comparison of control and treated groups shows the following features in the latter (1) rapid and definite improvement of subjective abnormalities and of the general condition (2) rapid and abrupt drop of bilirubinemia (3) certain number of recrudescences after discontinuation of cortisone and (4) lack of untoward effects of the hormone

(1) The rapid and definite improvement of subjective abnormalities and of the general condition has been a constant finding in the treated cases They rapidly regain appetite and are relieved of digestive complaints asthenia and malaise also if present vomiting and pruritus disappear The liver size diminishes and hepatic tenderness is soon no longer present

The treated cases regain their normal body weight around the four

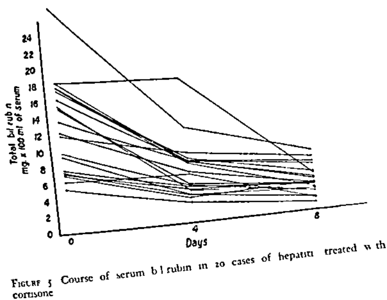


FIGURE 5 Course of serum bilirubin in 20 cases of hepatitis treated with cortisone

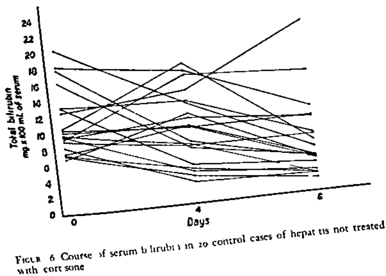


FIGURE 6 Course of serum bilirubin in 20 control cases of hepatitis not treated with cortisone

TABLE 1

PERCENTAGE OF REDUCTION ON EVERY FOURTH DAY FOR THE VALUES OF TOTAL SERUM BILIRUBIN IN RELATION TO THE INITIAL FIGURE IN TREATED AND CONTROL GROUPS

	Percentage of Reduction of Total Bilirubin	
Days of Treatment	*Treated (41)*	*Controls (41)*
0	—	—
4	55.4	25.2
8	72.7	51.8

individual values for 20 treated patients are compared with those for o controls (Figure 5 and Figure 6) The irregularity of the control curves (ascending 6 horizontal 7 and descending 8) contrasts with the uniformity of the treated curves (19 of the same type)

In only 6 treated and control cases could the bilirubinemia be controlled until it fell below 2 mg per 100 ml as most of our patients ask for their discharge before their complete recovery In the treated cases bilirubinemia reached a level below mg in an average of 18 8 days and in the controls of 15 8 days The 4 cases of recrudescence in the treated group are nevertheless included in the 26 here considered

(3) We consider as recrudescences those instances in which hyperbilirubinemia increases before it disappears In the control group only 1 case of slight recrudescence was observed

On the contrary in the treated group 4 patients showed recrudescences just before discontinuation of cortisone (eighth and ninth days) and the other soon after it was discontinued (tenth and eleventh days) In 1 of these patients the recrudescence manifested itself only by a slight increase in bilirubinemia not accompanied by other symptoms and similar to that observed in the control group In another the rise in serum bilirubin was concomitant to the reappearance of some symptoms and an increase in liver size this patient spontaneously recovered in 1 days

The other two recrudescences in the treated group were more severe In one marked digestive symptoms also reappeared which forced us to readminister cortisone they rapidly subsided and bilirubin dropped although less rapidly than the first time At the end of the second cortisone course (ninth day) and when the daily dose was 50 mg bilirubin rose without reappearance of other symptoms Therapy was stopped and the patient gradually improved until a serum bilirubin of mg was reached on the forty fourth day after admission

The fourth case of recurrence in the treated group corresponds to an instance of hepatitis with marked hepatomegaly and negative flocculation



FIGURE 8 Liver section of patient E.A. Biopsy performed before starting treatment with cortisone. Van Gieson × 90. Preserved trabecular architecture. Marked inflammatory infiltration in the portal spaces. Some of the cells appear necrotic.

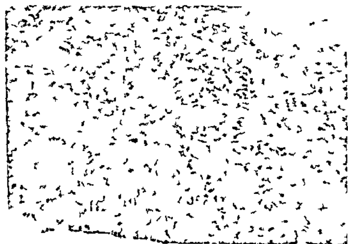


FIGURE 9 Liver section of patient E.A. Biopsy performed at the end of treatment with cortisone. Van Gieson × 90. Normal liver architecture. Slight inflammatory infiltration in the portal spaces. Absence of fat.

reactions The patient showed a recrudescence 3 days after termination of the cortisone treatment The hormone was again given for a total period of 65 days in a daily dose of 100 mg and when it was reduced to 50 mg bilirubin again rose After being in good condition for 36 days the patient suffered a relapse which was treated with a prolonged course of ACTH (5 days) The patient is now in good condition

(4) In none of the 39 patients treated with cortisone according to the scheme outlined above were any untoward effects observed The 2 cases who showed recrudescences and who received second courses of the hormone developed moonface but did not show edema ascites or other important complications

Among other observations worthy of mention is that the flocculation reactions followed a similar course during the hospital stay in treated and control patients In Figure 7 the thymol turbidity values are shown The course of the phosphatase values did not show significant differences between both groups

In 4 treated patients hepatic biopsies were performed before cortisone and from 9 to 11 days later in 2 marked improvement was observed This finding was seen also in 1 of 3 control patients in whom the biopsy was repeated 8 days after admission Comparison is difficult due to the small number of cases with repeated biopsies Nevertheless none of the liver biopsies performed in the treated cases at the end of the cortisone administration showed fatty infiltration In Figure 8 and Figure 9 the histo-

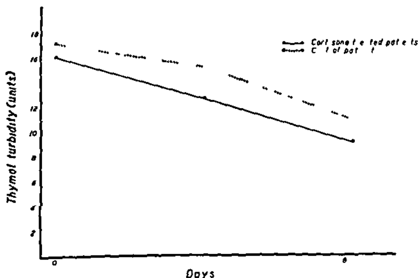


FIGURE 7 Course of thymol turbidity in treated and control groups

the hepatic cells Nevertheless our findings [1] in posthepatic jaundice with complete biliary obstruction seem to indicate that the steroids also have a direct effect on biligenesis which could account at least in part, for the drop of bilirubinemia in cases of hepatitis

Summary

A therapeutic trial of cortisone has been carried out in a group of cases of acute benign hepatitis selected according to certain characteristics *Treatment was given to 41 patients and 41 alternate ones served as controls*

The short treatment scheme used has not produced any untoward effects

The fundamental changes induced by cortisone were rapid and definite improvement of subjective abnormalities and of the general condition rapid and abrupt drop of bilirubinemia and some recrudescences and relapses

The routine steroid treatment of acute benign hepatitis does not seem advisable On the contrary cortisone appears indicated in severe and prolonged cases

The mechanism of the serum bilirubin drop following the use of cortisone is discussed

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logic appearances of the liver are shown at the beginning and at the end of the hormone treatment in one of these patients

Discussion

From the data given above it appears that the use of cortisone for a short period in the treatment of acute benign hepatitis induces the same fundamental changes as longer therapeutic schemes it has the advantage of being free of undesirable collateral effects

Our experience similar to that of others shows as positive findings the beneficial effect upon the symptoms and the rapid clearing of jaundice on the negative side a small percentage of recrudescences and relapses

It is very difficult to determine if cortisone really shortens the duration of the disease Some investigators among them Heilmeyer and associates[4] Evans and collaborators [9] and Huber and Wiley think that this is the case and the first 2 groups found statistical significance to their figures Nevertheless the observed differences are too small and the difficulties in assessing the real duration of acute hepatitis too great

We were unable to compare the actual duration of the disease in our groups The data regarding the beginning of hepatitis were very uncertain and in only 26 treated cases and controls could we make serial observations until the total serum bilirubin fell below mg per 100 ml

Our percentage of recrudescences (10 per cent) of which only half were important lies between the figures observed in other series (Heilmeyer and associates[4] 4 per cent Evans and collaborators [9] o per cent Siegenthaler and Suter[8] 5 per cent) Huber and Wiley[5] state that they were able to eliminate recurrences by the gradual suppression of the hormone nevertheless we followed the same procedure and have observed recurrences before stopping the treatment or reducing the dosage

We are now trying a slightly different scheme which uses smaller initial doses lasts a few more days and includes the use of ACTH at the end of the treatment

We do not think that the routine treatment of acute benign hepatitis with cortisone is to be recommended The uncertainty regarding the effect of the hormone on the duration of the disease the recurrences and relapses observed and the danger of the indiscriminate use of a drug with potential harmful effects like cortisone are against it On the contrary we are convinced that cortisone if used early and intensively is extremely valuable in the treatment of severe and especially fulminant forms of hepatitis It deserves also to be tried in the cases with a prolonged course

The rapid and abrupt drop of bilirubinemia caused by cortisone in acute hepatitis is interpreted as the result of the improvement of the excretory function of the liver This improvement would be caused by the slowing down of the inflammatory reaction and by the effect of the hormone on

GENERAL DISCUSSION

SHEILA SHERLOCK, M.D. (London, England): I would like to comment on Dr. David-son's paper.

We are in complete agreement with his regime of treatment and I think our results agree. The lines of treatment we use are first of all to search very carefully for any factor that might be diminishing liver function. The commonest is gastrointestinal hemorrhage but there are others like infection and the effects of surgery.

Secondly, all nitrogen in the intestine is eliminated. I am not going so far as to say ammonia in the intestine but I think we will all agree on nitrogen. We stop dietary protein, amino acids and ammonium salts. The diuretic diamox may induce coma in a very small number of people with cirrhosis. Calories are supplied by glucose or maybe starch hydrolysate is better. Certainly we do tend to get a diarrhea with oral glucose and dextrin may prevent this.

Following the observations of Faloon and Fisher we have replaced chlortetracycline by neomycin although we are fighting a losing battle with the National Health Service on account of the price. If the patient is kept on it for a year the cost is approximately $4000!

When considering the results of treatment in hepatic coma and judging from published results, the first thing to notice is the stage of coma described and this should always be specified. There is a great deal of difference between the results obtained when treatment is commenced in the precomatose phase than when it is commenced in deep coma.

The other point, as Dr. Davidson stressed, is that recovery must be clearly defined. I think it should be defined as ability to leave the hospital. If a patient recovers and then next week goes into coma again and dies, this patient should be struck off our recovery list.

Of 13 patients with acute virus hepatitis, 8 reached deep coma and 6 died. This emphasizes that deep coma in virus hepatitis carries a very ominous prognosis and I must congratulate Dr. Ducci on saving 5 out of 10.

In cirrhotic groups, acute cirrhotic coma must be separated from the chronic cirrhotic coma. By chronic I mean the patient in whom by-passing of portal blood is the main factor. The patient who develops neuropsychiatric symptoms after a portacaval anastomosis or who has a large spontaneous portal-systemic shunt will get better literally in spite of you. Of this group of 13 chronic cirrhotics 10 reached coma and none died. Of the acute ones 6 reached deep coma and 17 died. So we were saving 50 per cent of our patients with precoma or coma but only about one-quarter of those who got into the deep comatose stage.

The prognosis of hepatic coma depends really on early diagnosis

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was stopped the blood ammonia tended to return toward the control levels One patient had cirrhosis and was in coma but not deep coma He still responded to painful stimuli A gastric tube was inserted after the first 24 hours and he was fed 90 Gm of protein a day by tube

In addition to the peripheral venous blood determinations we determined ammonia levels in abdominal collateral vein blood There was a slightly elevated ammonia in the collateral vein blood over that in the peripheral venous blood during the first 3 determinations before neomycin was started When the neomycin was started it tended to fall to a level lower than that in the peripheral venous blood

Whether this means we are sterilizing the gastrointestinal tract and lowering the ammonia in blood coming from the gastrointestinal tract we are not certain but we think it suggests that Within 36 hours after therapy was begun the ammonia level fell to below 50 which is our top normal figure and continued at this level throughout the period of neomycin therapy

We were aware of the same problem Dr Sherlock was that this is an expensive form of therapy We had been using 12 Gm a day and we wanted to see what 8 Gm would do

Therefore we studied 3 patients receiving 8 Gm daily The first patient received neomycin for 48 hours followed by a fall in blood ammonia Following cessation of therapy the ammonia again rose After the second course he did not have a significant elevation in blood ammonia until the sixty fifth day of this study some 45 days after the neomycin was stopped This brings up a point which I will make later Of the other two patients one had impending coma and became stuporous and was fed largely by the aid of the dietitian He was not in deep coma Again in him the effect of neomycin we thought was significant Similar results were seen in the third patient

Our experience is not large enough to draw conclusions We have treated some 17 patients with neurological changes with acute disturbances such as acute bleeding or porta caval shunt surgery Of these we have lost probably 6 or 8 — I don t have the exact figures with me

We have a number of thoughts about this which may be worthwhile emphasizing First of all Dr Sherlock pointed out the important factor about antibiotic use in such patients — that probably the only effect of antibiotics in reducing ammonia formation is by sterilizing the gastro intestinal tract or by at least removing those organisms which produce ammonia As far as we know there is no other effect

There are several corollaries that exist One is that one needs to produce an intestine that is sterile or very nearly sterilized in order to get this effect Secondly there may be other compound in addition to straight ammonia or ammonium salts which may still be absorbed which will

whether there is a precipitant you can treat or not, and what the state is of the liver cells

Twelve patients with cirrhosis were first seen in deep coma and 8 died whereas were *first seen in precoma and only 9 of them died* The prognosis is much better if you start treatment early It is better if there is a clear precipitant such as hemorrhage Seven of 1 patients died showing steady deterioration but without a clear precipitant whereas only 3 died in whom gastrointestinal hemmorhage offered some prospect of control

Finally this emphasizes that prognosis depends on the stage of the liver cells and that is why it is so bad in virus hepatitis Five of 6 jaundiced cirrhotics died whereas only 10 of 3 of those without jaundice died I dont approve of Dr Davidson putting so much emphasis on portal hypertension as a cause of ascites The liver cells are equally important In those with ascites 14 of 2 died whereas in those without ascites only 3 of 12 died

This regime is obviously not perfect It is the best we can do at the moment Maybe we will all be able to think up something better next year I think this is a rational plan that offers some prospect of treating what has hitherto been a most fatal condition

WILLIAM W FALOON MD (Syracuse New York) With a new method of therapy I like others tend to be overenthusiastic Probably there are others around the country who have had more experience with neomycin than *we have had at this time This idea obviously is not original with* us It is a logical extension of the fact that ammonia derives very largely from the gastrointestinal tract

We have in our studies however attempted to do one thing that Dr Sherlock and Dr Davidson havent done and that is to continue *feeding protein* whenever possible while we are treating the patients with the feeling that only in this fashion can we evaluate the effect of neomycin by itself and insofar as possible free of the effect of lowering the protein intake

I think this can be best stated as being no panacea for hepatic coma The patients may still die despite this therapy they are awake perhaps a little longer but when the end comes they are just as dead as are the drowsy ones

We started with a relatively safe group of patients Most of these had slightly elevated ammonia levels which incidentally were not fasting determinations because we wanted to obtain nearly maximal blood ammonia levels *This we would not have obtained in the fasting state*

During the neomycin period when the patient was still eating a moderately high protein diet the ammonia fell and when the neomycin

was stopped the blood ammonia tended to return toward the control levels One patient had cirrhosis and was in coma but not deep coma He still responded to painful stimuli A gastric tube was inserted after the first 24 hours and he was fed 90 Gm of protein a day by tube

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There are several corollaries that exist One is that one needs to produce an intestine that is sterile or very nearly sterilized in order to get this effect Secondly there may be other compounds in addition to straight ammonia or ammonium salts which may still be absorbed which will

give an elevated ammonia once they are absorbed Such things might be glutamine or perhaps amino acids or other nitrogeneous substances Also neomycin does not protect against ammonium salts such as ammonium chloride

The lowering of blood ammonia which follows the administration of neomycin is dependent therefore entirely upon the ability of the liver or other tissues to remove ammonia If this function is damaged then the ammonia removal will not proceed as rapidly as one would desire The speed will depend probably upon the residual ammonia removal function Thus in the acute cases as Dr Sherlock mentioned the ones who have fulminating hepatitis the cirrhotics who are bleeding and in shock there will probably not be much fall in ammonia even though the neomycin does have an opportunity to work Also if the patient is severely ill and does not survive long enough for the lowering of ammonia, his coma may not be reversed

In agreement with Dr Sherlock we find the best results in the stable individuals and probably the poorest results in the obviously sicker ones

The effect of neomycin on blood ammonia will probably last only as long as the intestine remains sterile and in our experience this is a variable factor after the neomycin is stopped

A M Rappaport M D (Toronto Canada) I would like to comment on Dr Duccis paper

One finds very little in the literature about how cortisone affects the pathophysiology of the failing liver in coma and we are very grateful for his suggestions today but I am wondering whether there might be another a circulatory factor that enters into play

We have been able to produce experimentally the full blown picture of hepatic coma in dogs by inducing a severe ischemia in the dogs liver We formed an Eck fistula and 30 hours later we ligated the common hepatic artery The ligation of the common hepatic artery increased the ischemia of the liver leading to necrosis of the parenchyma In certain cases where the blood supply to the liver was very much curtailed the necrosis progressed and all the clinical signs of fatal hepatic coma ensued

In other cases where in the one or in the other animal good collateral supply to the liver was provided by nature we could first see the progress of hepatic coma to a certain state and then the regression of coma This went along parallel with the initial decrease and later increase of blood flow to the liver

I wonder if cortisone by reducing the swelling of the liver cells does open up the sinusoids and increase the blood supply to the liver I would be very much interested to hear if anybody has done blood flow studies

in patients suffering from acute hepatitis and treated with cortisone I realize how difficult it is

M BJORNEBOE, M D (Copenhagen Denmark) I would like to comment on Dr Ducci s paper in regard to Prednison treatment of chronic hepatitis

Short term treatment of women with chronic hepatitis has shown promising results in our country Long term treatment is now being tried Thygstrup and Winkler from the Copenhagen University Clinic have published 8 courses of Prednison treatment in 6 patients (1 man 5 women) and we have treated women in our clinic The result has in most cases been an increase in well being and appetite and — at the same time — a loss of weight and disappearance of ascites and edemas In 5 of 10 courses it was possible to stop treatment with diuretics The dose has been 20 mg Prednison a day

As I said this treatment looks promising Nothing that we have tried as treatment for these patients hitherto has been of any effect and we look forward to Prednison treatment as anyhow a good symptomatic treatment.

T C CHALMERS M D (Boston Massachusetts) A number of allusions have been made during the last 3 days to the controlled studies of the treatment of hepatitis conducted in Kyoto Japan 5 years ago this winter I want to emphasize that it was a large scale study in which a number of people were involved in Boston Kyoto and Washington and a lot of people I think can take credit or blame

There are a couple of things I want to comment on in regard to the study Dr Murray has reviewed the dietary aspects very well and I won t discuss them any further but I think the bed rest situation does deserve some further comment

In the first place I think we should emphasize the definitions used in comparing treatment effects In other words we had a group of patients who were kept at strict bed rest by rigid rules except for one trip to the bathroom per day and we compared them with a group of patients who were told that they could go to bed if they felt badly but that they could get up all they wanted to if they felt like it except for 1 hour in bed after each meal and except that they were confined to the hospital ward

It is a very important point that if the patient fe t sick he could stay in bed and could be waited on The bed rest was ad lib We forced nobody to get up and move around when he felt sick I have little doubt that if we had forced the sick people to march around as we have been quoted as having done we might very well have demonstrated some deleterious effects

When people ask me if I think this study applies to older people or to people who are sicker or to people who are not soldiers I say Yes because I think that if the older person or the nonsoldier has a more severe hepatitis and feels sick he can stay in bed and can be waited on but once he begins to feel better we can see nothing to be gained by forcing him to stay in bed no matter how deep his jaundice may still be

As a matter of fact there is a lot to be gained by letting him up because he avoids bed rest disease

It has struck me as I have sat here during the last days, that every time the treatment of infectious hepatitis has come up as a side issue as part of some other report — diet cortisone or what have you — it is mentioned that the patients were treated with strict bed rest I am sure it must run through your minds as it does through mine that now 5 years after the Kyoto study how could so many people still be treating their patients with strict bed rest when the advantages could not be confirmed by what seemed to be a carefully controlled study?

Obviously the answer is not easy to come by but there are a couple of comments that should be made One is the relationship of basic science or physiological observations to observations made on the human under clinical conditions The application of the oft quoted observations of Bradley that a patient on a tilt table has a decrease in liver blood flow when the table is tilted up to the treatment of the patient with hepatitis is a very good example of misapplication of basic physiological observations to clinical situations Certainly the patient sitting at his bedside for meals or playing poker all day or half a day is not very similar to the patient on the tilt table to whom lots of things happen including frequent fainting from a decrease in cerebral blood flow

I am sorry I missed the discussion in which physiological observation in animals versus humans was commented on I think the basic reason for the difference is that it is difficult to do a controlled study a carefully controlled study in humans However I would like to point out that during wartime situations when there is a large incidence of the disease under study a careful and well controlled study can be done and such a study is just as scientific as a study in which animals are manipulated

Furthermore given two scientifically comparable studies with conflicting conclusions one a study of rabbits in a case and the other of humans with hepatitis I cannot help but think that the conclusions of the latter study are more applicable to humans with hepatitis

ALFRED S EVANS M D (Madison Wisconsin) I would like to make some comments in regard to our experience with hepatitis when I was at the army hepatitis center in Munich Germany These observations were

made in conjunction with Colonel Robert S Nelson and Colonel Helmuth Sprinz.

The first point has been well brought out and that is the tremendous variability of hepatitis There is really no good way to predict the course of viral hepatitis on any objective data except perhaps the total serum bilirubin and in large groups this does give an indication as to the duration of the clinical disease but not in the individual patient

Secondly in garrisoned soldiers at least this is a mild disease in which 95 per cent recover almost irrespective of what you do to them

Thirdly the point about rest I am glad Dr Chalmers brought out a little better definition of what he calls rest It has been assumed that the treatment at the hepatitis center in Germany involved complete bed rest This was not so It was a modified program in which patients were allowed to be up and around the ward if they so desired or to remain in bed Under this regimen we had results similar to Dr Chalmers s This was a similar type of program

There was one group used as controls in the ACTH corti one study that were kept at absolute bed rest and by this we meant that the urinal and bedpan and commodes were all brought to the bedside and these patients did not get out of bed at all until jaundice disappeared In this group recovery time was no different from those who were allowed some freedom

Next in regard to exercise Dr Swift and Dr Gardner did a study at the hepatitis center in Germany some years ago in which they made the patients exercise rather violently — quite active calisthenics at different points in the level of serum bilirubin — and found that this made no difference in regard to the course of the illness if it were done when the serum bilirubin was under 3 mg per cent

Similarly a graduated exercise program begun in the jaundice period did not prolong the course except in patients with serum bilirubins that were over 3 mg per cent

In regard to coma of some 5500 patients whom we treated 16 developed coma and died These 16 cases in definite coma died irrespective of all therapy and included all cases in deep coma Six of these were treated with ACTH or cortisone with rather moderate scales of dosage as compared to Dr Ducci s This had no significant effect

Since then Colonel Nelson has treated 3 cases in definite coma with a schedule of at least 1 Gm of cortisone a day and 2 of the 3 have recovered I treated a similar case at Wisconsin who died In addition 1 other patient included in our report and who was in almost a comatose state recovered with the use of cortisone So I feel this is certainly one indication for a trial of cortisone therapy in large doses

Dr Ducci s very interesting data on the administration of oral cortisone

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Panel Discussion on Therapy, Course and Prognosis

CECIL WATSON *Moderator*

SHEILA SHERLOCK MD (London England) JOHN R NEEFE, MD (St Petersburg Florida) HANS POPPER, MD (Chicago Illinois) HECTOR DUCCI MD (Santiago Chile) T C CHALMERS MD (Lexington Massachusetts) HENRY G KUNKEL MD (New York New York) and M BJØRNEBOE MD (Copenhagen Denmark)

CHAIRMAN WATSON It is a little difficult to decide which question we should start with but there is one here that is rather general in character which I think might be good What are the complications of viral hepatitis I know Dr Sherlock has *some* information that would be very helpful concerning this question Let s face the problem first and get the complications listed and then perhaps we can have some discussion of them individually

DR SHERLOCK I would like to discuss what I have called complicated virus hepatitis The first complication is the fulminant form in which the liver suffers an overwhelming insult Death occurs within about a week if not then in my experience the patient recovers completely The reticulin pattern of the liver is unaltered amid all the chaos and if recovery occurs it is a complete recovery

The second complication is subacute hepatitis in which the patient shows a relapsing course develops vascular spiders splenomegaly edema of the legs ascites neuropsychiatric changes and is dead within six months — or if not then is left with the picture of posthepatitis cirrhosis I believe that when cirrhosis follows virus hepatitis it follows the subacute phase although not always a clinically apparent subacute phase The patient may not show all the clinical features of subacute hepatitis but can still develop the cirrhosis

The third complication is a prolonged picture of obstructive jaundice of which we are seeing a fair amount in England at the moment In the last couple of years I have seen 13 patients with a clear virus hepatitis history often with contact history who have shown predominantly obstructive features and who have *remained* with a *serum bilirubin* of greater than 14 mg for more than 14 weeks This was the group Dr Bockus was needling us on where there is difficulty in distinguishing

for short periods is quite similar to that which we obtained in a smaller controlled study. In our experience therapy with cortisone for as short a time as 1 or 2 weeks did not affect the total duration of the disease even though there was a marked drop in serum bilirubin. I understand this was also Dr. Ducci's experience. In longer therapy of 3 weeks it did have some effect on the course of the illness — a significant effect but of very moderate significance, one of perhaps 1 week.

Our relapse rate was 10 per cent under any schedule that we attempted even though we gave gamma globulin in an attempt to prevent the relapse in the wild hope that gamma globulin might find where the virus was or prevent a secondary viremia.

We have also had experience with cortisone and ACTH in moderately severe cases and feel that the progressive case with a serum bilirubin over 15 mg. per cent is sometimes benefited by cortisone therapy, but I think we should be quite careful in considering what we are doing with cortisone in this situation.

We are not, as far as anyone knows, killing the virus. We are not doing anything specific against this infectious process. At best we are only inhibiting the inflammatory reaction of the infectious process. It is true that this is a nice whitewashing agent through a poorly understood mechanism. The patient looks white and it makes the patient happy to see that he is now white and not yellow. It makes the doctor happy to see him somewhat whiter, but when one considers the total duration of the disease it does not make a really significant difference in a large group of cases.

So I think consideration of the use of cortisone should be reserved only for those cases in which you think the inflammatory process can be modified by cortisone to prevent death. In other words, for those cases in which vital processes are affected and not on any routine basis, because here I think relapses often make the problem very, very difficult.

We have some evidence in liver biopsies, Dr. Rappaport, that the use of cortisone does in fact diminish the inflammatory reaction of the hepatic cells. I have no data on the blood supply, but coded liver biopsies given to our pathologist, Colonel Sprinz, of cases treated with and without cortisone demonstrated when uncoded a very definite diminution in the inflammatory lesion in the liver biopsy. Three of these 10 cases so examined also had quite marked fat infiltration.

So in conclusion I would recommend great caution in the use of ACTH and cortisone on any routine basis. I think Dr. Ducci's results are very nice indeed.

with vascular spiders, splenomegaly edema ascites and so on and 4 of them had subsided to apparent inertia

In the remaining 13 patients with inactive cirrhosis there was a period after the hepatitis in which they were able to work normally and seemed to all intents and purposes normal Seven of these are still in that stage and have been followed up to 5 years We know they have cirrhosis we think it developed after the hepatitis but it is not causing them any particular trouble and we don t know when it will Eight of them after a mean of 5 years have shown hematemesis implying portal hypertension and in general portal hypertension is much more prominent in this group than it is for cirrhosis associated with alcoholism After 8 years 8 of them show ascites and liver failure

We believe as Bloomfield believed that after the original attack there can be quite a long latent period before trouble begins

I have divided cirrhosis into 5 speculative groups the clinical type the fulminant the subacute the prolonged obstructive and the group with prolonged flocculation tests I have expressed these in terms of patho logical lesions In the fulminant necrosis is prominent regeneration is nonexistent reticuloendothelial reaction in the liver is slight and bile retention is minimal The prognosis is usually very bad although as I have said if recovery occurs it is complete

The subacute liver shows necrosis regeneration, and reticuloendothelial reaction all continuing together with a minimal of bile retention The prognosis is bad Either the patients die at that stage or they progress to a cirrhosis

In the prolonged obstructive liver necrosis is plus minus Parenchymal damage is very slight Regeneration is nonexistent and reticuloendothelial reaction is very slight perhaps just one plus Bile retention is very prominent The prognosis is good

The prolonged positive flocculation test group is the type that is some times called wrongly chronic hepatitis The term chronic hepatitis must imply cell damage whereas this group doesn t usually show this feature There is no necrosis and no cell regeneration and reticuloendothe lial reaction is three plus whereas bile retention is not seen These patients are not jaundiced and ultimately recovery is complete In summary the prognosis depends on the amount of liver cell damage that exists and on whether or not the acute attack has resulted in destruction of the reticulin structure of the liver

CHAIRMAN WATSON Dr Sherlock has admirably introduced the question of the possible late complication of hepatitis and the question of transi tion to cirrhosis Dr Popper I know has been very much interested in the question of the type of cirrhosis which develops following viral hepa

extrahepatic biliary obstruction This is the condition that Dr Watson and Dr Hoffbauer described as cholangiolitic hepatitis I have avoided that term because like others I dont like invoking the cholangiol or disease where I am not quite sure of the nature of the cholangioles and whether or not they are in fact diseased so I would rather call this *complication hepatitis with a prolonged obstructive stage* In my experience this is followed by complete recovery I dont think primary biliary cirrhosis is a sequel of this type

Finally there are various benign sequelae such as the posthepatitis syndrome which is failure to gain weight nasty ache over the liver—that one little drink and you feel dreadful and so on This is a syndrome that is particularly common in the medical and nursing profession and I am sure all of you clinicians here will bear me out on this Symptoms are usually I think of a functional nature related to the fear of developing cirrhosis Then there is a group in whom cellular infiltrations remain in the portal of the liver for a long time with associated positive flocculation tests Thymol turbidity for instance may be positive for 6 or 7 months Recovery is complete

These are the patients I have seen with what I think *is posthepatitis* cirrhosis How do I know this lesion followed hepatitis? Well I dont All I know is that these people had a disease which was clinically indistinguishable from virus hepatitis and who developed cirrhosis In 5 of them I had an original biopsy which showed the picture of virus hepatitis There were 22 men and 1 women in the group the numbers being loaded by the military forces during the last war and that is why there are more men than women The mean age was 34 in fact most of them were less than 30 It is a younger age group than that of cirrhosis associated with alcoholism None of my patients took excessive amounts of alcohol

In the original attack 8 had erum hepatitis and 26 were *infectious* of whom 16 had a contact history and 10 of these during military service The original attack in 11 you might say was classical in that they were jaundiced less than a month and they *made an uninterrupted recovery* In 12 I classified it as severe they developed either very deep jaundice or ascites or were away from work for many months Eleven had a relapse So I think we can say that posthepatitis cirrhosis may follow classical hepatitis but it is more common after the severe type or after a relapse There were 12 deaths in the series 7 with hematemesis and liver failure

I have divided the patients with posthepatitis cirrhosis into two groups the "active and the inactive The inactive showed very little except perhaps a *palpable* liver the biochemistry was negative

There were 11 patients in the active group At the end of 3 years 2 of them were dead 5 were continuing along in an active stage that is

with vascular spiders, splenomegaly edema ascites and so on and 4 of them had subsided to apparent inertia

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titis and so I am going to ask him to devote a little time to that if he will

DR. POPPER As Dr Watson just mentioned we have been very much interested in the morphologic pathways by which a lesion which is characterized in its original stages by a single cell necrosis may proceed into cirrhosis In a relatively small number of cases of the fulminant type of hepatitis to which Dr Sherlock made reference earlier a massive necrosis takes place There great parts of the parenchyma collapse and postnecrosis cirrhosis results

This is a well established course of events and several names are given to this condition — coarse nodular cirrhosis toxic cirrhosis postnecrotic cirrhosis — all characterized pathologically by great variations of the picture throughout the liver Part of the liver parenchyma may look almost normal as a matter of fact on liver biopsy in such an area a perfectly normal picture may be obtained whereas in other areas broad connective tissue bands are present

Although there are relatively few cases in which one clearly could demonstrate this course of events by liver biopsy I think most clinicians and pathologists will accept this pathway

However much more difficult to understand is the type of pathway which starts with single cell necrosis associated with cellular inflammation around the single necrotic cells which Dr Gall discussed previously How can such a lesion proceed into cirrhosis?

Briefly I want to describe some experimental evidence for such a progress of single cell necrosis into cirrhosis from studies with G Kent A Dubin and C Bruce If rats are put on an ethionine containing diet two changes develop both of which are similar to what we would see in human viral hepatitis during the spotty necrotic stage single cell necrosis accompanied by single cell regeneration and a marked interstitial cell reaction This interstitial cell reaction consists in part of inflammatory cells and in part to our great surprise of cells derived from the bile ductules We do not mean the ducts even the smallest ducts in the portal tracts but the so called canals of Hering cholangioles or whatever other name is given to ductular structure within the lobular parenchyma and not within the portal tracts

In such rats (and we made similar observations in the human but could study them better in rats) this ductular cell reaction has some similarities with an inflammatory reaction These ductular cells sometimes greatly resemble mesenchymal cells Upon injection of India ink into the bile ducts of such rats one can demonstrate between cells looking like mesenchymal cells bile ductular channels That means what may look like a mesenchymal reaction may actually represent proliferation of the

smallest bile ductules the epithelial cells of which appear disorganized and are frequently intermixed with unquestionable inflammatory cells

In connection with what has been discussed before I would like to add that cortisone suppresses completely the ductular and the inflammatory reaction whereas the liver cell damage persists After injection studies of the blood veins indicate that in the subacute stage of the ethionine intoxication the blood flow is impaired by the described interstitial reaction The administration of cortisone probably abolishes the blood flow interference in the experimental rat although liver damage exists

In this stage of diffuse single cell necrosis a fibrosis sets in along two pathways One pathway follows collapse of the liver cells and subsequent collagenization of the reticulum framework The second — and this was a big surprise to us — is the formation of basement membranes along these ductular cells which resemble mesenchymal cells

These basement membranes eventually aggregate and thus develop into septa which dissect part of the lobules and thus produce regenerative nodules Finally typical cirrhosis — either diffuse septal or postnecrotic — follows a process starting with single cell necrosis and is never associated with massive cirrhosis In the coarse nodular (postnecrotic) cirrhosis serum gamma globulin is markedly elevated just as in human post necrotic cirrhosis

In conclusion the rat observations demon trate the possibility of a slow but active lesion which is characterized by histologic changes similar to the human viral hepatitis developing into a diffuse septal type of cirrhosis without any episode of catastrophic breakdown of hepatic function

CHAIRMAN WATSON I wonder if one of you gentlemen would like to comment further on this question I think we ought to treat the question of cirrhosis in relation to hepatitis now Dr Bjørneboe I know you are particularly interested in this

DR BJØRNEBOE I would like to take this opportunity to make a few remarks concerning our findings in Denmark on the relationship between the incidence of hepatitis and fatal cirrhosis of the liver

To my knowledge the first to demonstrate anatomically the development of cirrhosis of the liver after acute hepatitis was Krarup by means of the liver aspiration biopsy technique of Iversen and Roholm

To illustrate further this connection between hepatitis and cirrhosis of the liver I shall utilize Danish medical statistics which are probably among the most reliable in the world

I shall consider hepatitis first Since 19 8 this disease has been notifiable in Denmark Returns are sent by the local general practitioners to the Medical Officers of Health who in their turn send them to the Depart

ment of Medical Statistics of the Ministry of Health This method is naturally associated with certain errors Not all cases are notified Thus, Rysing found in 1946–1947 that 84 per cent of all the cases of hepatitis diagnosed in Copenhagen during a 6 month period were notified There are, however no reasons to suspect that this inaccuracy varies from year to year Incorrect diagnoses must also occur i e silent gallstones and cancer but particularly in such a characteristic disease as hepatitis, incorrect diagnoses are probably rare

Figure 1 shows the incidence of hepatitis in Denmark month for month from 1928 to 1956 All that is known concerning the incidence of hepatitis in the years immediately prior to 1928 is that the incidence was greater than in 1928 but exact figures are not available Since then there were two waves with maxima in 1933 and 1943 respectively and in addition the characteristic seasonal variation for the disease with maximum in the winter months and minimum in summer These seasonal variations are most pronounced in those years in which the incidence of hepatitis is greatest

Figure shows that the sex distribution among adult patients during the years has remained unchanged at approximately 50 per cent males and 50 per cent females As regards the age distribution on the other hand a striking change has occurred as adults comprise an increasing proportion of the total number of patients (Figure 3 and Figure 4) In 1930–1935 nearly 40 per cent of the patients were adults while since 1945 approximately 70 per cent of the patients were adults In the forties we assumed that this condition was connected with the hepatitis virus prevalent at that time However the age distribution has not altered since As in poliomyelitis the alteration in the age distribution might be attributed to the fact that increasing hygiene in Denmark causes the population to encounter the virus infection later and later in life The great undulations in

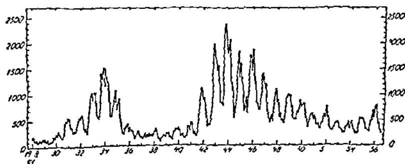


FIGURE 1 Hepatitis cases per month in Denmark 1928–1956

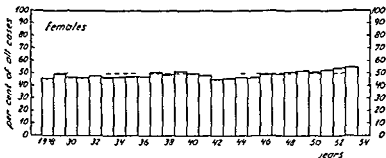


FIGURE 2 Sex distribution in hepatitis patients over 15 years of age

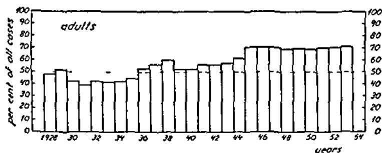


FIGURE 3 Age distribution in hepatitis patients (adults over 15 years of age)

the incidence of hepatitis are more difficult to explain Variations in the virulence of the virus and the resistance of the population are possible causes The existence of various types of virus is also a possibility These variation can hardly be called epidemic waves in the ordinary sense of the word as they extend over a period of years

Figure 5 shows the incidence of cirrhosis of the liver as the cause of death in Denmark since 1931 From 1931 onwards the death statistics of the Ministry of Health are based upon death certificates practically all completed by physicians In this connection considerable errors must be anticipated as regards the diagnosis It should however be stressed that a great number of the patients particularly in towns die in hospitals where the possibilities of establishing an accurate diagnosis are relatively good *

The figures show that as is already known there was a great increase in the incidence of cirrhosis of the liver as a cause of death in the forties Further it will be observed that since then this cause of death has remained at a higher level than previously

* During 1955 87 per cent in towns 62 per cent in the country died in hospital

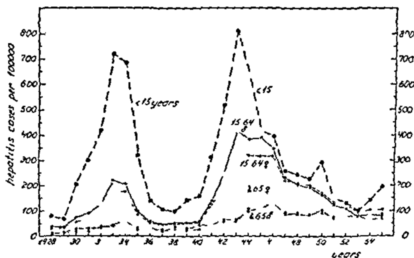


FIGURE 4 Age distribution in hepatitis patients.

I have attempted to control this condition in a single hospital (The Municipal Hospital Copenhagen *) by reviewing all the autopsy notes from 1928 until 1955 The notes are reviewed with the macroscopic description of the liver in mind It will be observed from Figure 6 that the principal change which has occurred after the forties is an increase in the number of cases of postnecrotic cirrhosis I am of the opinion that this at least partly explains the fact that in this country as a whole more cases of cirrhosis are recorded as the cause of death now than previously Postnecrotic cirrhosis is more frequently recognized clinically than portal cirrhosis which is often first discovered at autopsy Therefore a greater incidence of postnecrotic cirrhosis should be more probably reflected in the death statistics than an increase in the incidence of portal cirrhosis

The epidemic of hepatitis in the thirties is not reflected in the mortality from cirrhosis while in the forties and later there is undoubtedly a connection between the incidence of hepatitis and the mortality from cirrhosis Figure 7 and Figure 8 show that it is not the mortality from cirrhosis among males which follows the hepatitis graph, but the mortality from cirrhosis among females

The observation that hepatitis in the forties and later was more frequently followed by cirrhosis than in the thirties is probably connected with the fact that older age groups are now attacked by hepatitis and this

* My thanks are due to S Petri M.D Chief Pathologist the Pathological Institute the Municipal Hospital, Copenhagen for permission to use this material Some of it has already been published

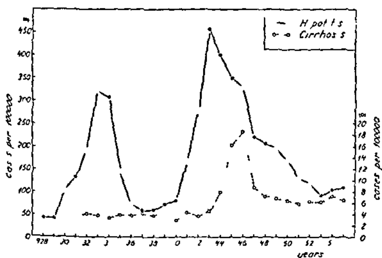


FIGURE 5 Frequency of cirrhc is cf the l ver as cause of death as compared with frequency of hepatit s

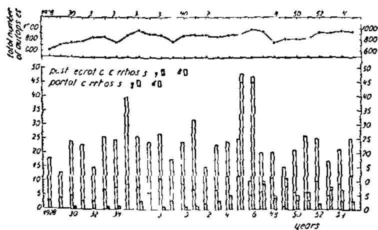


FIGURE 6 Incidence of portal c rrl s and postnecrotic c rrhosis in autopsy record Kommunehospitalet C p nh g n 928– 955

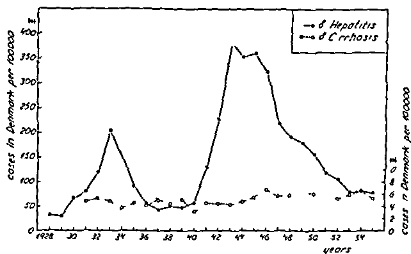


FIGURE 7 Incidence of cirrhosis of the liver as cause of death in men as compared with frequency of hepatitis in men over 15 years of age

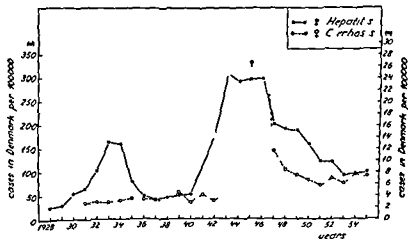


FIGURE 8 Incidence of cirrhosis of the liver as cause of death in women as compared with frequency of hepatitis in women over 15 years of age

disease runs a more serious course in the older age groups* This has previously been stressed by Ryssing concerning the epidemic wave in the forties but the condition still seems to hold true

This seems to indicate that the etiology of cirrhosis in females differs from the etiology in males This supposition is supported by an investigation by Hamtoft of the mortality from cirrhosis in recent years in Denmark According to this investigation approximately half of the male patients suffering from cirrhosis are alcohol addicts while only very few of the female patients are alcohol addicts

I have considered myself justified in drawing attention to these Danish figures for the incidence of hepatitis and the mortality from cirrhosis although they have been published in part previously They demonstrate several conditions which are difficult to investigate in countries which have no corresponding medical statistics

The figures show firstly that the disease hepatitis occurs in waves which extend over several years These are not epidemic waves in the ordinary sense

Secondly these figures show that hepatitis in Denmark has altered in its character from a disease of children to a disease which on the whole attacks adults This is not only a condition which characterizes the occurrence of hepatitis in the forties but seems to persist

Thirdly the figures appear to show that this age displacement implies that more females now die from postnecrotic cirrhosis In males portal cirrhosis still dominates and here addiction to alcohol seems to be an important factor in the development of cirrhosis

CHAIRMAN WATSON I am very glad that Dr Bjørneboe has presented these interesting statistics to us They really are somewhat reminiscent of the experience in our own hospital the University of Minnesota Hospital over the years where we have had a much smaller incidence of cirrhosis in alcohol addicts than is true in the Minneapolis General Hospital in the same city or in the Ancker Hospital in St Paul There I think the experience is comparable to that in any municipal hospital with from 80 to 90 per cent of the cases being alcoholics Our statistics at the University Hospital would run between 40 and 50 per cent — closer to 40 per cent alcoholics — and these are mainly males

The idiopathic or posthepatitic cirrhotics are mainly females or at least much more frequently females than males We think we do see exactly the same picture in males but not anywhere near as frequently

We have not been impressed with the incidence at or past the meno

* It should be pointed out that 5 to 6 per cent of all who died in the home for old people in Copenhagen (De Gamles By) in 195 –1955 proved at autopsy to have cirrhosis of the liver

pause as we have been with the incidence in young females just beyond the menarche I think many more of our cases have been in the younger age groups than in the later ones With that exception our experience rather reflects what Dr Bjørneboe has said

I agree that there is a very important sex factor here I think in any consideration of cirrhosis one has to consider the type of material one is dealing with Certainly in a large municipal hospital we have mainly one type of cirrhosis the type Dr Davidson was talking about earlier In my hospital where much of our material comes from rural areas we have much less alcoholism and much more of the type of cirrhosis that Dr Bjørneboe has discussed

Dr Neefe I wonder if you would care to add some comments about this problem because I know you have been very much interested in it for a long time

DR NEEFE I think it is an interesting problem because there is still a great deal of uncertainty and conflict in many people's minds about the exact relationship of viral hepatitis to cirrhosis

There are some people I believe who still deny that there is any relationship at all However in three attempts to make a systematic survey of persons who have had hepatitis well documented hepatitis who have been followed up some 4 to 8 years later * the three groups all came out with almost identical results namely that when a large number of persons who had hepatitis are studied after that interval there is no greater incidence of chronic liver disease in those who had jaundice than in the control groups I think that would be the conclusion from that study Would you agree Dr Watson?

CHAIRMAN WATSON Yes certainly from the standpoint of any statistical analysis that is what one has to say about Dr Zieve's study He did have cases of cirrhosis in the patients who had had hepatitis and 1 in the control group and this difference was not statistically significant

Perhaps we should keep in mind again with relation to the sex incidence that the great majority of the individuals in this study were males in all three of these large groups

DR NEEFE That is the next point I want to emphasize The results in these studies really seem to be in conflict with the experience of almost anyone who has seen hepatitis in the population at large I think we have to admit that these cases exist because Dr Sherlock has just shown a

This has been done in about as thorough a way as has ever been possible by Dr Zieve working at the Minneapolis Veterans Hospital in that area by Dr Turner and his group in New Orleans and by a group of our own in Philadelphia

group and I think anyone who has seen liver disease in all sorts of people has seen patients in whom he felt quite sure that hepatitis must have had something to do with it

As Dr Watson mentioned these studies were limited and their results can be applied only to the fact that they were nearly all men and also men in a certain age group for the most part

I think it is probably fair to say that there is one other very important factor i e that there is a difference not only in the people who get these diseases but in the agent itself

One thing that was most impressive that I am sure Dr Murray will remember was one experience we shared somewhat jointly with a carrier whose blood was put into a group of volunteers at one stage of his course and which produced a disease of average severity in the volunteers About 6 months later that volunteer himself developed acute hepatitis which is an unusual circumstance among carriers About 6 months after recovery from his hepatitis blood again was taken from that donor and was again given to volunteers This was about the most vicious virus present in the blood taken after the donor had jaundice that I have ever seen at close hand Two of the volunteers came down with acute hepatitis at about the same time and both went into coma within 24 to 48 hours One died the other recovered We were worried because 3 other people had this circulating in them They had globulin and did not come down Whether or not that was the reason they didn t come down I don t know But here was a virus or viruses present at 2 different times in the same individual I hardly believe it just happened that there were two unusually susceptible patients among the second group There must be some change at times in the virulence of the organism or perhaps this man had acquired still another virus at some stage in the game so that I think we do have to keep in mind that perhaps some of the differing results may depend on a differing etiologic agent

CHAIRMAN WATSON Dr Ducci what is the impression with regard to this whole problem as far as your material in Chile is concerned? You have a great amount of material there in terms of both hepatitis and cirrhosis

DR DUCCI Regarding Dr Sherlock s classification of complicated cirrhosis I agree with her in general It is very similar by the way to the one we published several years ago in *Gastroenterology*

I think there is one type at least in our experience that is missing in that classification and that is the recurrent type of hepatitis We see that once in a while Those patients do not develop a true cirrhosis but develop what Marchand called many years ago nodular hyperplasia this is a distinctive type different from the true cirrhosis and different from the subacute type

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There are three types of studies in this regard One is the careful follow up of patients who have had hepatitis In these studies as pointed out by Dr Neefe no association has been demonstrated but this does not mean that it could not have been missed Secondly there are several poorly controlled studies of the occurrence of jaundice in the past history of patients with cirrhosis which seem to indicate a positive association The final approach is the one pursued by Dr Bjørneboe which I must admit is impressive From the epidemiological standpoint he has shown a definite association

Let s assume then that an association is some day established Does this mean that viral hepatitis causes cirrhosis Does this establish cause and effect?

Those of us who believe that a cause and effect has not been established might explain the hypothetically demonstrated association by saying that there is a disease of unknown etiology — and I am now referring to non alcoholic cirrhosis — which occurs in about 1 to per cent of the population which is usually fatal but may be found incidentally at autopsy and which has a variable course often beginning with an episode of jaundice which may be confused with acute viral hepatitis Recurrent episodes of jaundice might also be characteristic of this chronic metabolic disorder of the liver

Finally I think it is unwise for us to keep talking as though viral hepatitis had been established as the cause of cirrhosis for two reasons first because it leads us not to strive to search for the real cause or the most common cause of nonalcoholic cirrhosis and secondly it makes the doctor scare his patient (whether purposely or unconsciously) and thus create a great deal of posthepatitis disability

Finally whenever we see a patient who develops a disease that looks like viral hepatitis and who goes on to die of cirrhosis I think we must remember the fact that coincidence occurs all the time in medicine and that the only way to be sure we are not dealing with coincidence is to have a statistically controlled series

CHAIRMAN WATSON It is quite clear that there is a little disagreement among our panel This is an extremely difficult problem and I have said many times recently that we really are at an impasse Until we can get a method of easily detecting the virus in the liver or in the blood or excreta or at least of proving that an individual had a viral hepatitis in his past history I am afraid we are not going to get very much further

Just in the past literature I think the evidence in favor of the thesis that viral hepatitis causes cirrhosis is overwhelming — even from the pathological literature alone If one goes back to the splendid monograph of Bergstrand in the large epidemic in Sweden in around 19 4 as far as I can

In regard to cirrhosis and hepatitis I know this is a very complicated subject but I would like to call your attention to the fact that we very seldom see a patient in the acute stage of the disease who after a period of apparent well being develops hepatitis if we can follow him right from the start

The great majority of our patients with cirrhosis that we consider related to hepatitis have cirrhosis right from the start It is the same thing that happens with chronic nephritis and acute nephritis The patients with chronic nephritis often start as chronic nephritics right from the beginning and the same thing happens with cirrhosis and hepatitis I can recall only one patient we saw during the acute stage who after a period of several years of apparent well being developed true cirrhosis of the liver

Dr Kunkel The only thing that I would like to comment on is perhaps a little too controversial to bring up here but possibly it will stir up some arguments

In studies that are being undertaken to determine the incidence of posthepatitis cirrhosis do not liver function tests in control series often confuse the picture In those instances where a young navy or army man who is in excellent health suddenly develops an episode of acute jaundice in an area where many other cases exist and then continually has difficulty from that day forward for a period of 5 or more years can one disregard this sequence of events just because normal control series show individuals with positive liver function tests Most of us have seen such cases and have been convinced that cirrhosis in rare instances follows what at present must be considered typical attacks of acute infectious hepatitis Three out of approximately 400 navy men who we observed in their acute episode during World War II fall into this category and continue to show abnormalities of liver function at the present time

Dr Chalmers I am concerned about the problem of the man who gets drunk and falls down flat on his face Is the cause alcohol or gravity or was he tripped I am quoting from Dr Boyd s superb talk last night because I couldn t help thinking all the way through it that it had tremendous application to the problem we are discussing at the moment — the problem of cause and effect

Does viral hepatitis cause cirrhosis of the liver? I think we have to approach this question in a systematic way We have to ask is there an association? Has an association been established — a significant association? I do not refer to the single patient whom all of us have seen who started off with what might be viral hepatitis (but for which we have no proof) and who has gone on into cirrhosis but from an over all viewpoint is there a statistically significant association?

had any jaundice and no acute attack and who yet present themselves with clear cut evidence of a long standing liver disease

I have seen this happen interestingly enough in a mother and daughter Dr McKenna who is here I am sure will remember these two people The mother developed this disease at about the age of 43 and died after running a course of about 5 years altogether the first 3 years of which were nonicteric About 4 years later her daughter appeared at the age of 19 with the same disease in the same evolution and with the same end result

The question in my mind has always been is this another form of viral hepatitis That they both had cirrhosis was not a question They both had the type of hepatic test response that one sees in hepatitis but that is not specific. It is a very puzzling group to me The one had a positive lupus erythematosus phenomenon and yet at post mortem the pathologists did not see anything that they would diagnose as lupus erythematosus

CHAIRMAN WATSON Before we go on, I would like to ask your indulgence to make just a few remarks about a point that I was reminded of when Dr Sherlock was speaking namely this question of the prolonged hepatitis in which there is little or no evidence of hepatocellular functional impairment — what Dr Hoffbauer and I chose to call the cholangiolitic type and which Dr Sherlock spoke of as the obstructive type

As was said earlier we did not use the term cholangiolitic from a histologic point of view because we did not feel we could define this as a histologic lesion We used this term only to express the concept that the smaller biliary radicals within the liver were functionally impaired

The thing that impressed us in those cases was the fact that as the biopsy was looked at i e the histology very little was seen — really no good evidence of obstruction sufficient to account for the jaundice That was really the main reason why we did not like to use the word obstructive because we could see no evidence of obstruction

Certainly there was no evidence of obstruction of larger bile ducts The cholangiograms which have been made in many of those cases both prior to our publication and subsequently were entirely normal In other words we were clearly not dealing with any large bile duct disturbance

In looking at the histology very little of a mechanical obstructive nature was found to explain this disturbance yet here was clear evidence that a large amount of bile at least many of its elements was getting back into the blood — regurgitation of bile if you want to think of it in that way

And so our concept was simply that this was a functional disturbance

see it is impossible to escape the belief that many cases of cirrhosis resulted in that epidemic of hepatitis

It has seemed to me that the main argument centers about what type of cirrhosis—not whether cirrhosis results but in what type of cirrhosis If one goes on to the later work in more recent years Dr Bjørneboe referred to the studies of Krarup and Roholm who as he said were the first to show transitions from epidemic hepatitis on a serial biopsy basis to the development of cirrhosis

This sort of thing was also done in more detail by Axenfeld and Bras There have been a number of studies of that type I have seen a sufficient number of cases myself that are so convincing and of that general character that I at least cannot escape the belief that viral hepatitis may result in cirrhosis

To me the question is why doesn t it happen more often? What is the X factor in these individuals which causes the cirrhosis to develop occasionally? It is perfectly clear that it does not develop in the vast majority of cases

I was deeply interested in Dr Kunkel s observations of the increased susceptibility to stilbestrol in 5 of his cases Although these had shown no historical relation to hepatitis nevertheless the fact that they did have this increased susceptibility to estrogen I think is an interesting lead and one that ought to be followed up very carefully

I have had the impression in a number of cases of this type that they had profound endocrine disturbances before they had any evidence of liver disease This is a very difficult thing to be sure of as of course you are not there at the right time to make the investigations you would like to but at least from the historical standpoint one gets this impression rather frequently

There is fair literature on this point although I must admit it is not as objective or as convincing as one would wish I think we need more studies of this general character to determine whether in the female there are at times endocrine factors which predispose to the development of cirrhosis after the individual has had viral hepatitis

I am sure I agree fully with Dr Chalmers that just the fact that we may believe that viral hepatitis occasionally gives rise to cirrhosis should not in any way prejudice us against trying to find out about other factors

Are there other thoughts about this aspect of the problem from the panel before we go on to some of the other questions?

DR NEEFE I think we ought also to bring into this whole picture at this point the other type of clinical story and that is the patients (commonly females and probably quite similar to some of those we have been talking about) who on their very first visit to the doctor may not have

and perilobular structure and duct for the interlobular structure in the portal tracts

We would interpret the cellular infiltration around proliferated ductules in the subacute stages (I don t know whether Dr Watson will go along with this) not as the cause of the lesion but rather as its result produced by prolonged regurgitation of bile through these ductules into the periductular connective tissue which becomes inflamed because of the seepage of the bile

Dr Watson has compared this process very well with lower nephron nephrosis where also without morphologic evidence necessarily being present the permeability of the tubules is abnormally increased It is our opinion that what has been called pericholangiolitis is really a response to the regurgitation rather than the cause of the regurgitation and we are a little supported in this opinion by the observation of similar alterations after approximately the same time in established extrahepatic biliary obstruction We therefore assume that bile seeping from the cholangioles into the interstitial tissue for 4 or 5 weeks produces secondary inflammation Whether this inflammation finally causes fibrosis which adds a mechanical obstructive feature we can only speculate

Now to the anatomic features As I mentioned earlier we have been extremely impressed with the role of the ductular cells We think ductular cells are of great importance in hepatitis and many other conditions We may not recognize them because they look like mesenchymal cells and we need special techniques such as injections to be sure of their nature Another method is staining for alkaline phosphatase which selectively can be demonstrated in these cells

These ductules we believe develop from liver cells It is usually stated in embryology textbooks that the liver cells develop from bile ducts and the latter from ductular cells We now know on the basis of modern embryology that that is not the case — to the contrary from the liver cell anlage ducts and ductules develop secondarily We therefore assume that one response of the liver to damage is the transformation of liver cells into ductular cells which may sometimes appear not as ductular cells but as inflammatory cells And furthermore that as additional response to the injury the ductular cells become abnormally permeable permitting regurgitation of bile and inspissation of biliary material within the ductules from loss of water This results in bile plugs which provide a mechanical factor in this intrahepatic cholestasis This hepatic response may be elicited by injuries of varied etiology including viral infections drug sensitivity or poisons

CHAIRMAN WATSON We have some clinical problems to which we must address ourselves at least for a time

an injury of the smaller biliary radicals an increased permeability so that bile was able to leak back into the blood This was by no means a new concept Actually it went back to Minkowski Aschoff talked about the relative weakness of the lower portion of the bile capillary the so-called ampulla Aschoff many of you will remember spoke of it as the Achilles heel of the biliary tract

Whether this was the point of leakage or not we were unable to say because we had no means of localizing this with the microscope The fact that there was evidence of leakage of bile back into the blood without any evidence of obstruction was what prompted us to use the term cholangiolitic thinking of it simply in a functional sense—a functional clinical sense—the two things combined because these patients usually do have a fairly typical clinical appearance in addition to the laboratory findings

I simply wanted to say that more or less to set the record straight so that there would be no question about our having used the term cholangiolitic from a histologic point of view

Dr Popper you might perhaps like to comment further about that

Dr Popper I was very much stimulated by the paper by Dr Watson and Dr Hoffbauer and we were for many years interested in getting a little more clarification on this question of the alteration of cholangioles or ductules

There are great difficulties for the pathologist Dr Watson clearly pointed out in his paper and re emphasized here that morphologically one cannot demonstrate a characteristic lesion in the portal tracts or within the lobular parenchyma in these cases

We have some possible pieces of information to add to what Dr Watson has said First if one studies histologically a large number of such instances and recently this opportunity arose in the many cases of intrahepatic cholestasis with little or no liver cell impairment which follow thorazine administration one is impressed by the frequent absence of changes in the portal tracts in the acute stage the eosinophilic exudation and edema of the tracts so characteristic for the cases with drug sensitivity is transient and often missed Also within the lobular parenchyma only bile stasis is noted

After 4 to 6 weeks of jaundice cellular infiltration in portal tracts and around proliferated cholangioles or ductules is regularly seen We would prefer to have a clear cut nomenclature We have omitted the term cholangioles because some have used it for bile canaliculi and some even for the interlobular bile ducts We propose therefore (and I know Dr Sherlock agrees) to use the terms bile canaliculus for the structure which is surrounded by liver cells ductule for the small intralobular

yet he appeared to recover from it In the last few years of his life he had little or no trouble with his liver and he died of an entirely different disease

I mention this just because it is an extreme case Most of them don t do this well and perhaps this in itself indicates a different etiology I am sure Dr Chalmers would say that I think there is a very broad spectrum in this type of case but we don t seem to be able to influence them one way or another

DR NEEFE I don t have anything to contribute to this type of case other than the fact that it strikes me that one of the most important things faced with that situation is to once again sit down and make quite sure one has the correct diagnosis Thus there have been many patients who started out with what looked like a hepatitis and who failed to recover in the usual time

I have seen a number of these cases in army hospitals because they were so unfortunate as to get jaundiced at the same time that a lot of other men did they were carried along indefinitely as having chronic hepatitis Several such patients had a few gallstones in the common duct

I think whenever there is an undue prolongation of what seems to be hepatitis a careful search ought to be made for other important causes I was wondering if Dr Sherlock would perhaps consider a biopsy in a patient of this type as well as other diagnostic studies

I have one other point that I am not sure is important but several patients of this type (and I know others who have encountered them) are found to have associated conditions that perhaps are not causative but perhaps at least assist in the perpetuation of the hepatitis I am thinking of such things as amebiasis which often is not recognized because of marked clinical activity I can recall one or two such patients who had an unusually long course who failed to recover completely and who were found to have amebas in the stool After treatment with appropriate agents they seemed to go on and clear up

So I do think it is important to look around for other things that might be contributing to the picture

DR BJORNEBOE I would like to commen on Dr Neefe s remarks I want to say that under these circumstances I think an exploratory laparotomy should be avoided

We had many cases of chronic hepatitis in the 1940 s and in the beginning we were not familiar with this disease Several of these patients were operated on for purely diagnostic reasons and many of them died after the operation I think it is very important in these cases to avoid laparotomy

What is the treatment for a patient who 5 to 10 months after an acute episode of hepatitis has a slightly tender liver with a slight elevation of bilirubin and/or slight bromsulphalein retention

Dr Sherlock would you care to cast the first stone

DR SHERLOCK I really haven t been given enough information I would have thought that with a still positive bromsulphalein test and an increased serum bilirubin level the patient was probably developing a posthepatitis cirrhosis If he was then I would expect one or two other associated clinical features such as the development of a few vascular spiders and splenomegaly If he had both of those things I would be pretty confident that he was developing a posthepatitis cirrhosis

What am I going to do about it The answer is very little I don t know what to do There is no particular cure for cirrhosis I don t think I would influence the outcome by putting him to bed I am just going to be moderately reassuring and hope that as in many of my cases the cirrhosis will subside to an inactive form

The alternative diagnosis is that he just has positive flocculation tests If that is so he would not have the serum bilirubin and the bromsulphalein change In the patient with only positive flocculation tests I would anticipate complete recovery I am afraid I would not be very active about this

DR CHALMERS I think I would agree completely with what Dr Sherlock has said and I would be inclined to reassure the patient and myself because *I can t think of anything more positive to do*

CHAIRMAN WATSON I thoroughly agree with that I feel that we are really very helpless in the face of this sort of situation but it has been remarkable to me that there are such striking individual variations Certain of these people do make a very good recovery even after several years of intermittent low grade recurrences like this general type

I am thinking of a doctor whom I saw first more than twenty years ago He had had what appeared to be infectious hepatitis in a mild epidemic and his jaundice persisted for some time He had low grade hepatocellular abnormality and then apparently he got over it Then he had a recurrence Eventually he had a biopsy which showed chronic hepatitis with definite cirrhosis and fibrosis The cholangiogram was normal

He had at least three prolonged episodes of low grade icterus with some mild tenderness of the liver and yet he got over each one after a number of months Finally he was able to return to his practice and he died of a coronary thrombosis

I am sure this disease went at least 10 years and perhaps longer and

progressive disease which to me does not fit very well with the term postnecrotic

I realize I am very much in the minority because apparently most people like the term postnecrotic and it has come into wide usage

Dr Kunkel Dr Chalmers s statement reminded me of an instance where we had in our small collection of patients with what we called post hepatitis cirrhosis one individual who we thought was very typical of the group He was a boy approximately 15 years old We followed him for 3 years with this diagnosis although in retrospect he did not have a history of a typical acute attack of hepatitis Suddenly he developed neurological manifestations of Wilson s disease It is just another illustration of the many pitfalls one encounters

Dr Bjørneboe In answer to Dr Chalmers s remarks I would like to say that in Denmark we had a lot of postnecrotic cirrhosis during the late 1940 s You could exclude alcoholism in these patients as a cause of cirrhosis You could exclude malnutrition As a matter of fact in Denmark overnutrition is much more common and more dangerous At the same time there was a very high incidence of hepatitis This I think is a good point for the existence of the posthepatitis type of cirrhosis

I should like to add that I think that it is not without significance that these reports of posthepatitis cirrhosis come largely from countries like Switzerland Sweden Norway and Denmark small countries with a very good and well organized National Health Service where follow ups are easily done You can find 100 per cent of the cases again I think that is one reason why we have been able to publish so many papers on this subject

Chairman Watson That is an excellent point

Here is another question What are the criteria of recovery to be used in allowing a housewife to return to normal duties following infectious hepatitis

Dr Ducci That is a tough question I think you have to judge by the clinical aspects of the patient especially hepatomegaly jaundice clearing of the urine and normal bilirubin The usual mistake is to wait for the flocculation reactions to become normal Those are absolutely unrelated to the eventual recovery of the patient

When bromsulphalein and bilirubin are normal and all clinical signs have disappeared I think she may return to active duty

Chairman Watson Would you allow her to return if her bromsulphalein was 10 per cent?

Dr Sherlock May I add one other diagnosis that tends to be confused with this particular situation and that is congenital hyperbilirubinemia Many young people with this condition get a little nauseated and ill are noticed to be a bit jaundiced and are diagnosed as having hepatitis and are treated as chronic invalids for months In fact they are suffering from the essentially benign condition of congenital hyperbilirubinemia, but then of course they will have the flocculation test changes

Dr Chalmers I am delighted to see the conversation going in the direction it is taking because it is just the effect I was after in emphasizing that I am not convinced that viral hepatitis can cause cirrhosis The pendulum has swung too far in one direction Too often we hear the term posthepatitis cirrhosis applied to all cirrhotics who do not drink alcohol

I think we should prefer the terms postnecrotic or idiopathic or nonalcoholic cirrhosis I would add another etiology we have encountered Occasionally patients with Wilson s disease will have one or many episodes indistinguishable from acute or chronic viral hepatitis ending in a fatal cirrhosis with or without neurological signs Yet they have a specific metabolic abnormality of their liver which certainly is in no way related to viral hepatitis

Dr Sherlock Do you think you ought to use the term virus hepatitis ?

Dr Chalmers I think the evidence for the viral etiology of acute hepatitis is much more impressive than the evidence for viral hepatitis as a cause of cirrhosis

Chairman Watson In order to leave the question entirely open I am quite willing to call these patients idiopathic I don t like the term postnecrotic for several reasons I think practically all cirrhotics have some necrosis at one time or other It is a matter of degree how massive it is

Furthermore the term postnecrotic has the great disadvantage in my mind at least of indicating that there was one episode of necrosis in the past and that it is now a static condition just related to the scarring which has resulted from this single episode of massive necrosis acute atrophy the same as healed acute atrophy whereas many of the patients with this picture that is generally called postnecrotic cirrhosis start in rather insidiously and build up slowly It is often a slow progressive relentless disease in our experience It isn t at all like what you would expect if there had been one single episode of severe hepatitis with massive necrosis and then a static condition of postnecrotic scarring It is a

progressive disease which to me does not fit very well with the term postnecrotic

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When bromsulphalein and bilirubin are normal and all clinical signs have disappeared I think she may return to active duty

Chairman Watson Would you allow her to return if her bromsulphalein was 10 per cent?

DR DUCCI It depends on the dosage used

CHAIRMAN WATSON Say 5 mg per kilogram

DR DUCCI I would We have a 14 per cent upper limit of normal

DR SHERLOCK I agree fully with Dr Ducci that one should not wait for flocculation tests

CHAIRMAN WATSON Do you include the cephalin flocculation test too?

DR SHERLOCK Yes

CHAIRMAN WATSON You dont worry about 3 plus or 4 plus?

DR SHERLOCK No I think a lot of people are made into chronic invalids *by having weekly flocculation tests done when they are apparently* recovering quite normally from virus hepatitis

CHAIRMAN WATSON I am a little more conservative about the cephalin flocculation test If a person has 3 plus I would be inclined to watch him for a while before I made a final decision in the matter I fully agree with respect to the thymol turbidity which I think is much more influenced by immune mechanisms than by liver cell injury but I would not be too happy about allowing full activity if the Hanger test were still 3 plus or 4 plus

DR DUCCI I would like to add a word about the problem I fully agree with what has been said before about the diagnosis of chronic hepatitis *I think this diagnosis has to be made with great caution* I have seen so many cases misdiagnosed as chronic hepatitis with nonhemolytic jaundice or common duct stones hyperbilirubinemia and many other things

The only thing I disagree with is the influence of rest I have seen several patients who several months after an acute attack of hepatitis have a slightly enlarged spleen a mild hyperbilirubinemia who were put to bed for some time and who completely recovered I think we have to test at least the inference of rest on these patients

CHAIRMAN WATSON *I want to add a word about this matter of rest be*cause Dr Chalmers discussed the careful study carried out in Kyoto Japan in which I was much interested

I think those findings are entirely valid and the conclusions are right but I dont believe this can be translated directly to allowing an individual

to return to a full day s work with all the stress that may be entailed I think this is an entirely different thing from sitting at the bedside walking around as Dr Chalmers said playing poker and doing things like that

I think some people may actually mislead the doctor perhaps subconsciously They are very conscientious and want to get back to their activities I have had the experience a number of times of having patients indicate to me that they felt perfectly well when I knew that they had considerable residual liver disease which was still much too active for them to return to a full day s work

I think we must differentiate between permitting them to return to their full activity and ad lib activity under careful supervision I doubt very much whether strict bed rest is essential I am not sure at all that position is important but I do believe we must think about this matter of stress It seems to me that we must try to get these patients to avoid mental stress and fatigue If they become fatigued then I think they are likely to get into trouble

I would like to ask if anyone disagrees with that point of view I would like to hear any other comments about it and to know whether there is unanimity on the part of the panel This is a very important practical question

Here is a question addressed to Dr Sherlock We recently gave a chronic cirrhotic 10 Gm of methionine intravenously and within several hours the patient was in deep coma for the first time What is the mechanism of action of methionine in this instance?

Dr Sherlock Dr Watson I think you were one of the first people to describe methionine as being toxic to patients with liver disease In 1947 I believe you mentioned a patient who went into coma after being given methionine

Chairman Watson I am afraid I didn t know it was more than a casual relationship

Dr Sherlock This is a very real association in a small proportion of patients with liver disease The question here concerns intravenous methionine Our experience was that this was relatively innocuous We gave a number of patients who had cirrhosis oral methionine in a dose of 10 Gm a day and usually by about the third day some would deteriorate mentally and show signs of impending coma Then we repeated this in 5 of those patients who deteriorated with oral methionine using 6 Gm of intravenous methionine In 4 of them nothing happened the fifth showed some deterioration about 4 hours after we stopped the methionine

Our feeling was that the methionine was toxic but only in the intestine I am a little surprised that this response was so clear cut with the intra venous route

Incidentally with oral methionine our patients went into coma without any change in the blood ammonia level This has been confirmed by some French workers and adds evidence to the point I would like to make that we ought not be satisfied entirely with the ammonia toxicity theory of hepatic coma but that there must be other means by which hepatic coma can be produced Certainly the methionine toxicity seems unrelated to ammonia

CHAIRMAN WATSON Does anyone on the panel feel that methionine has any place now in the therapy of liver disease Since there is no response I guess the answer is No

In the previous panel discussion a question arose as to the occurrence of infectious hepatitis viral hepatitis or at least inflammatory hepatitis in patients with pre existing cirrhosis—alcoholic cirrhosis I know Dr Popper is interested in this and Dr Bockus asked him to comment but he was out at the time He has some interesting information that I will ask him to give to us now

DR POPPER I am afraid I have no information Dr Watson This is a point that confuses me very much We all know that in a patient with cir rhosis of nutritional and other types acute episodes of jaundice develop characterized by laboratory evidence of hepatocellular damage and mor phologic evidence of liver cell injury In some instances this takes the character of the so called hyaline or Mallory bodies produced by coagula tion necrosis primarily in the nutritional type of cirrhosis

In all these instances of acute hepatocellular injury morphological and clinical in a patient with established cirrhosis the question arises whether this represents an episode of hepatitis specifically of a viral type grafted upon a pre existing cirrhosis

I was interested in looking for some morphologic evidence for the as sumption of an episode of viral hepatitis in a patient with nutritional cirrhosis and fatty metamorphosis I have never seen a case of this type of cirrhosis with the well known morphologic signs of viral hepatitis I have asked many of my friends to show me such a case but so far I have not been successful However I am aware that as long as we cannot demonstrate the virus we can never be sure of the diagnosis of viral hepatitis

I can think of two possibilities to explain the absence of morphologic changes of viral hepatitis in nutritional cirrhosis with fatty metamorphosis One is that the pre-existing morphologic changes may obscure the charac

teristic features of viral hepatitis Dr Watson just took exception to the term postnecrotic cirrhosis I fully agree with him that necrosis occurs in almost every type of cirrhosis I once suggested the term postcollapse cirrhosis because the really basic feature is the massive collapse But in lieu of a better name available at this time I refer to postnecrotic cirrhosis and in this form I have seen in several instances in the areas outside of collapse the characteristic morphologic features of viral hepatitis despite the presence of cirrhosis I observed the acidophilic bodies (Councilman bodies) acute single cell necrosis with accumulation of lymphocytes and other mononuclear cells as well as lipochrome pigment So I am a little doubtful as to whether in other types of cirrhosis like the fatty one these features can be cured

The second possibility is that a patient with pre existing nutritional cirrhosis is in a way protected from viral hepatitis This is of course very fantastic and I am in no way willing to assume it I am again asking my friends for specimens of a nutritional cirrhosis in which you can demonstrate the classic morphologic picture of viral hepatitis

Dr Chalmers I hate to say this but if one believes that viral hepatitis causes cirrhosis then one would naturally expect that the patient with cirrhosis is immune because of his previous exposure

Chairman Watson He might be immune on many other bases I think I have seen two cases but I can t prove it histologically because I haven t studied it in this way From a purely clinical point of view I think I have seen two instances in which an individual who was an alcoholic and who had a rather static so called alcoholic cirrhosis developed an acute attack of jaundice without acute alcoholism and had features very strongly suggestive of infectious hepatitis and then recovered I fully realize that is by no means conclusive evidence

Dr Popper There is of course in the cirrhosis enough functional features present which make it possible to produce liver cell breakdown We may get in to the cirrhosis liver cell breakdown and hepatitis if you want to call it that very frequently but morphologically I cannot call it viral hepatitis

Dr Ducci I think that to discuss this point we have to be very precise If we call hepatitis any inflammatory reaction most cases of cirrhosis have it Usually the clinical problem on the ward for the residents is that of a patient with alcoholic cirrhosis and an episode of jaundice they always ask Is this a case of hepatitis on top of cirrhosis or is it an icteric episode of the cirrhotic condition?

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In none of these cases of alcoholic cirrhosis with jaundice have we found the chemical features of acute hepatitis The enzymes are normal or only very slightly elevated Thymol turbidity is slightly increased and in most cases thymol flocculation is negative We don t find the features that we find in acute hepatitis So we think jaundice in cirrhotics can exist without superimposed hepatitis and of course it is usually a very bad prognostic sign

CHAIRMAN WATSON I quite agree It is usually due to the underlying disease *Of course in many of these alcoholics it is due to an added bout* of acute alcoholism which produces a much more severe jaundice and is very dangerous

Before we adjourn it is my pleasant privilege to be the spokesman for all the members of this conference everyone who has been here in thanking the Henry Ford Hospital for a very fine occasion I do want to express our gratitude *to the Board of Trustees to the staff of the Henry* Ford Hospital and to the Arrangements Committee

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